The Journal of LABORATORY and CLINICAL MEDICINE

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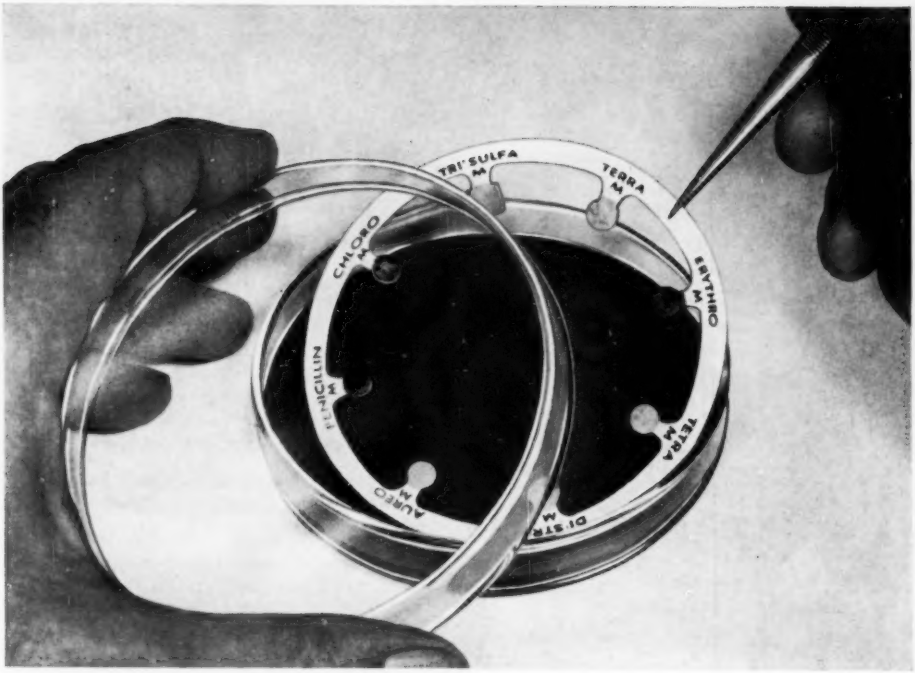

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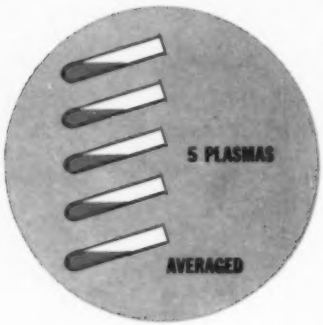

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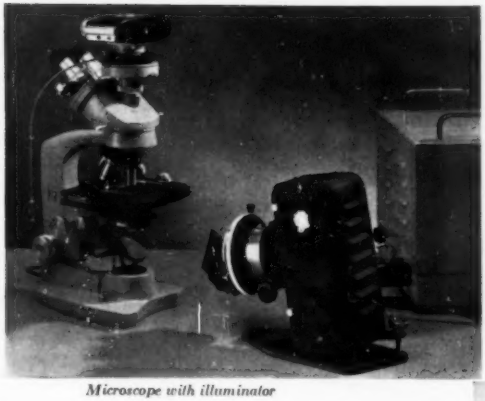

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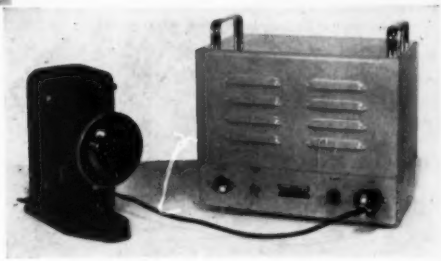

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Quality Control in Clinical Chemistry was a featured presentation earlier this month at the American Association of Clinical Pathologists meeting in Chicago. The use of freeze-dried reagents *like* Versatol and Versatol-A was recommended for routine daily control.

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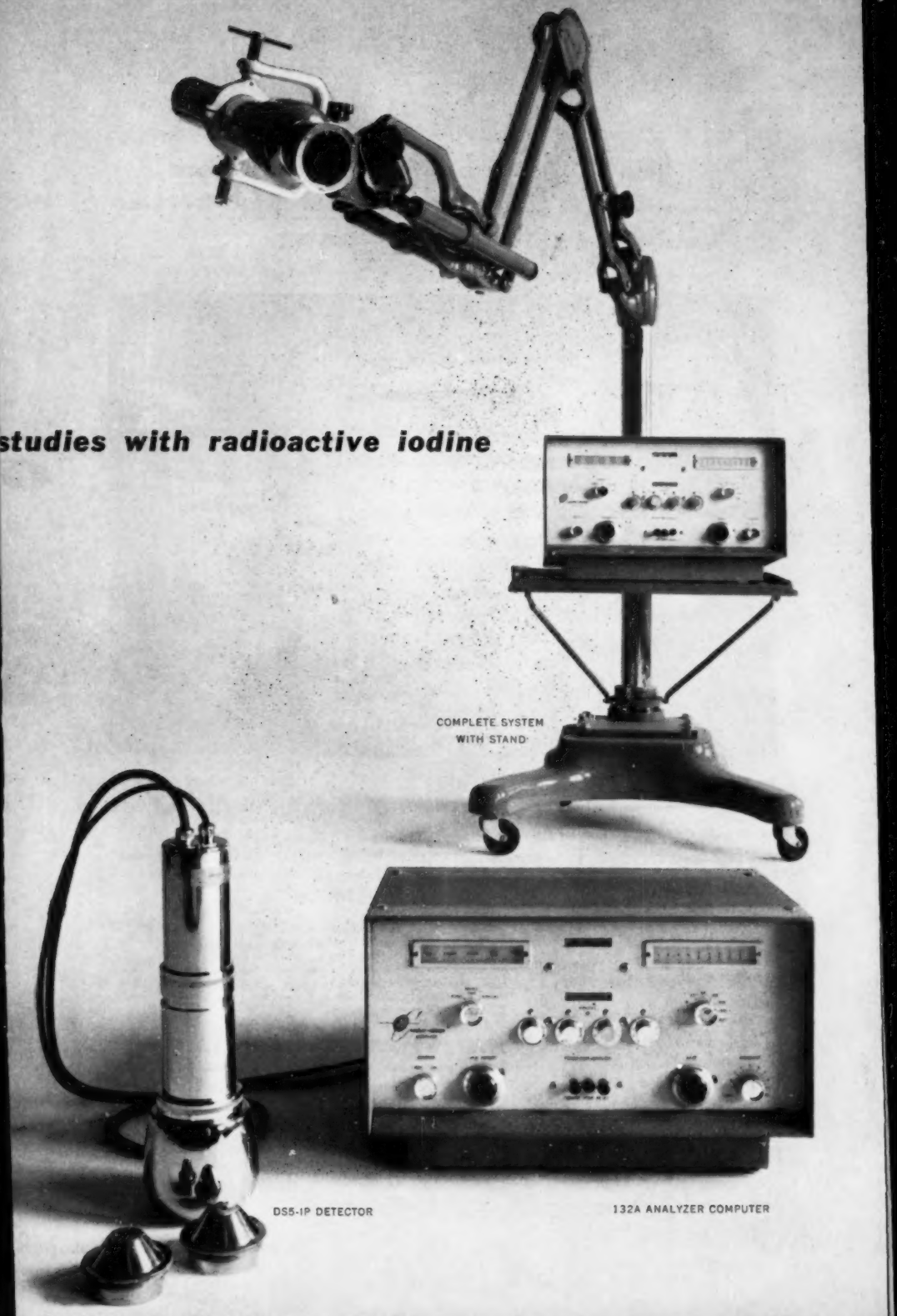
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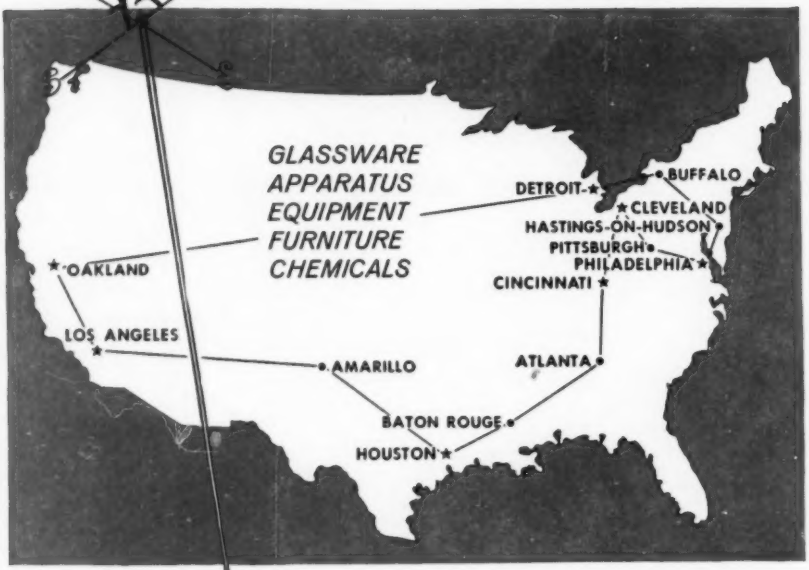

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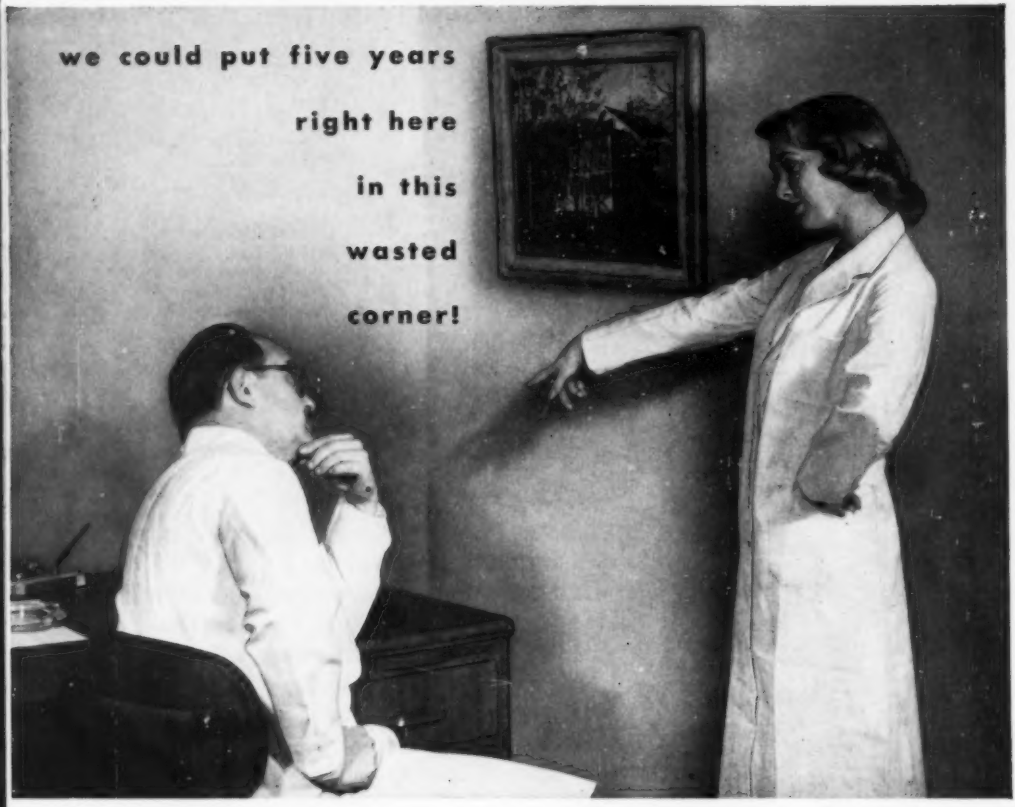

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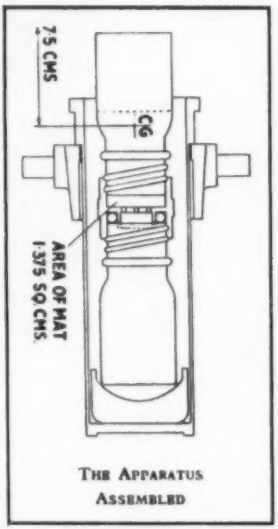

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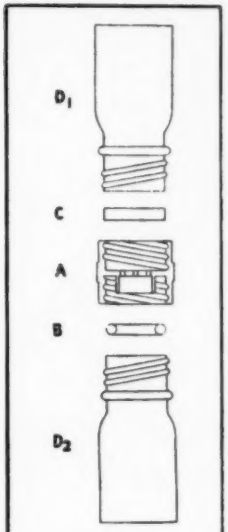

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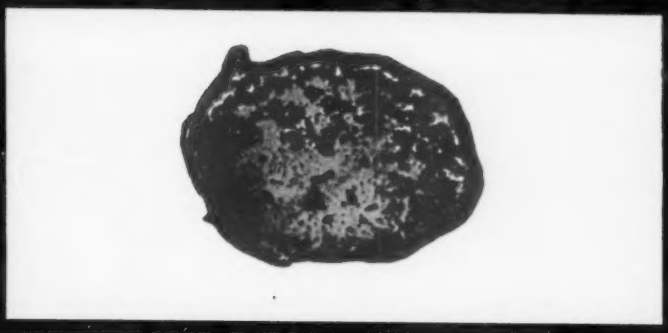

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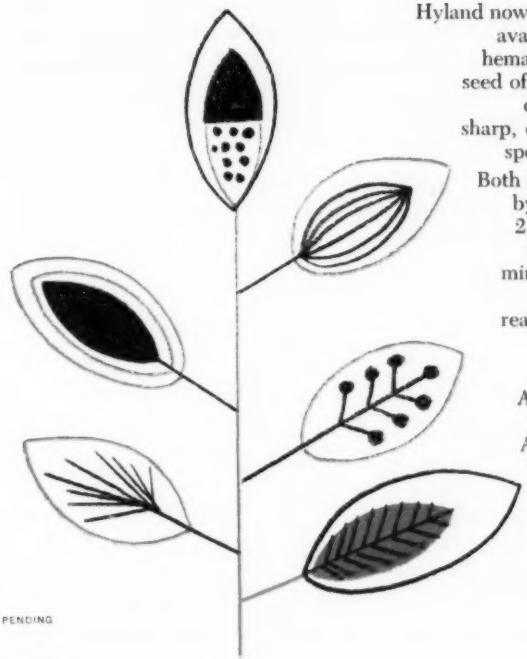

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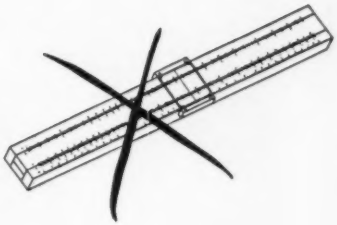

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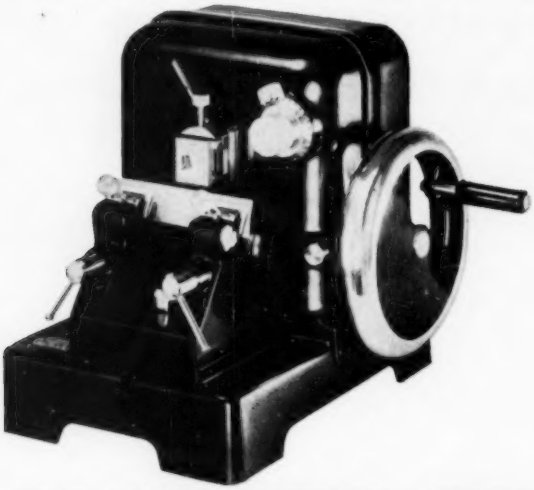

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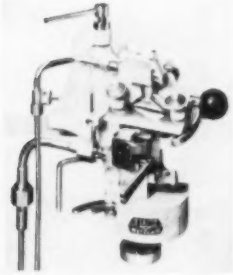

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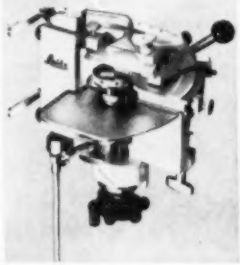

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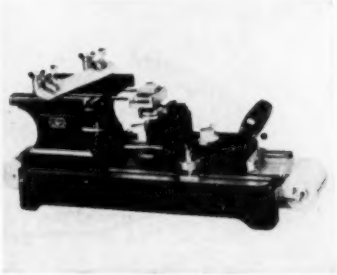

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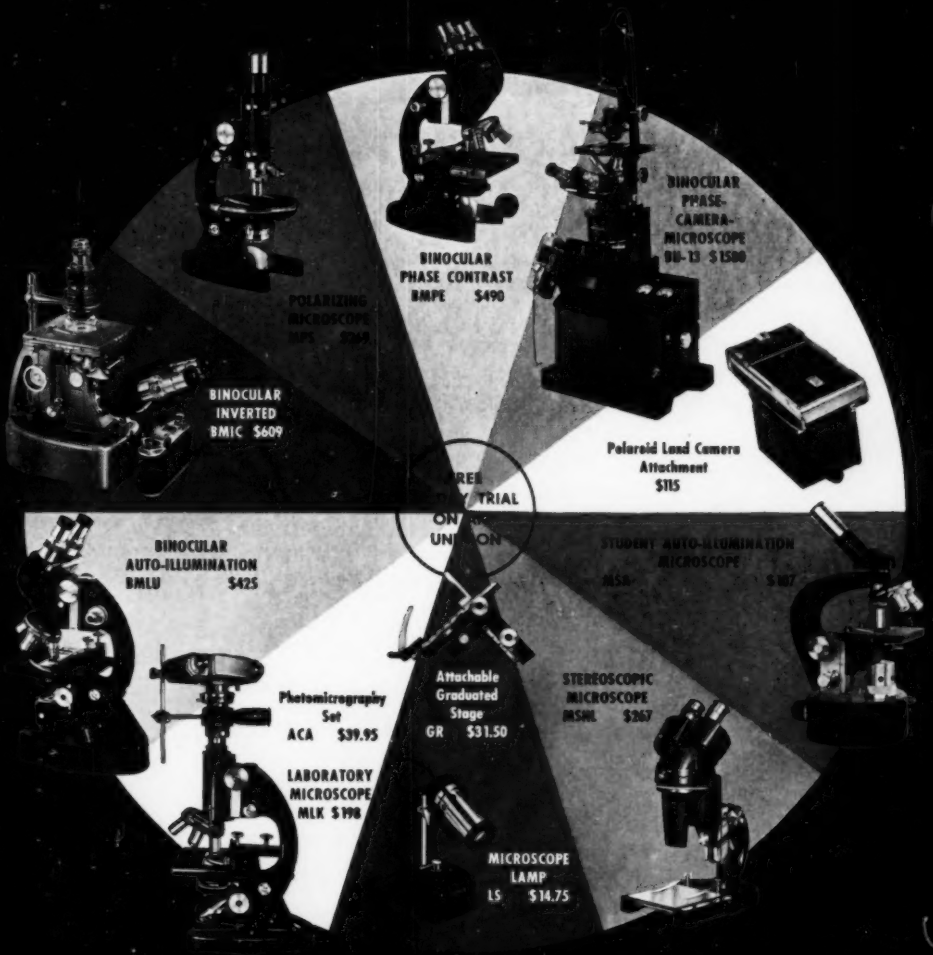

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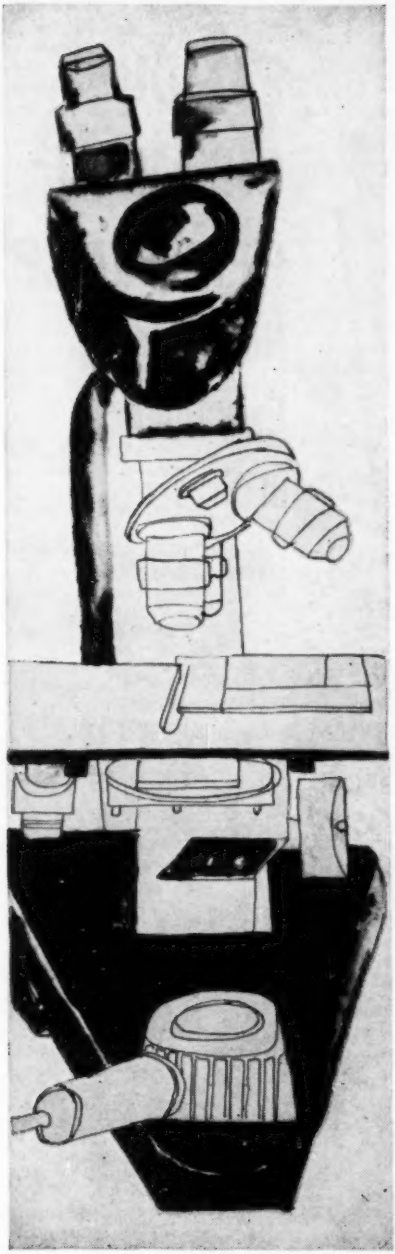

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Microelectronics

Ever hear of the Diamond Ordnance Fuze Laboratories? It's a Department of the Army agency in Washington. To support DOFL, the average citizen shells out the federal tax on, let us say, several gallons of gasoline a year or a little tobacco. Since a fuze is a device which times an explosion to blow up his enemy, he probably wouldn't mind the expense if it were explained to him. But, *mirabile dictu* and happy day, prospects brighten that the piddling investment will pay off beyond the dreams of avarice!

DOFL has spawned "microelectronics," the shrinking of electronic assemblies to 1/100 normal size. DOFL became involved through the proximity fuze program, which requires very small and exceedingly rugged components. We are involved through certain products we make. Microelectronics may be bigger than both of us. It may make possible the placement of electronic devices inside the body, for diagnosis or for replacement or repair of human organs with electronic equivalents. It may permit a new directness in the study of the central nervous system.

The theme of microelectronics is that if you want environment-immune, highly "intelligent" circuitry that can handle problems of logic and fit into a tenth of a cubic inch of space or so, you quit at an early stage of the design thinking of transistors, diodes, capacitors, resistors, and such. Instead you think of the circuit as one or more plates half a millimeter thick and fabricated as intricately as necessary out of various conductive, semi-conductive, and dielectric materials disposed among the three dimensions of each plate.

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Alkylsulfonyl from different viewpoints

A gentleman of Oak Ridge called to our attention a paper (*J. Chem. Soc., 1957,* 2420) which, in the course of examining 29 different alkylsulfonates, reports ethyl methanesulfonate as one of four which effectively inhibit a transplanted rat carcinoma. Previously Haddow and Ross (*Nature, 177,* 995) had reported that this particular compound has the striking feature of greater efficiency when administered orally in aqueous solution (20 mg/rat/day) than by daily injection of the same solution. The sequence of ideas which led Haddow and Ross to this finding is interesting.

The initial idea was that chloroethylamines are potential tumor-growth inhibitors. The second idea was to use the compound 2-chloroethyl methanesulfonate as a reagent for chloroethylating an amino group under mild conditions. The third idea was to run this reaction *in vivo* instead of *in vitro.* This actually worked. 2-Chloroethyl methanesulfonate proved an effective inhibitor in the rat both intraperitonally and orally, and it induced no significant depression of bone marrow. The fourth idea was to write 2-chloroethyl methanesulfonate as $CH_3SO_2OCH_2CH_2X$ and vary the X from Cl to something else. With X as fluorine, cytotoxic activity persisted. The big surprise must have come with X = H, the unsubstituted ethyl methanesulfonate, the one that works better orally. This shifted the spotlight to the other end of the molecule, where dwells the methanesulfonyl group.

Our heads buzzing, we gave up trying to understand the basic biochemical issues. Our role is that of storekeeper; we must remember that. And what methanesulfonates did we have in the store? We had *n-Butyl Methanesulfonate* (Eastman 4671). (There had been requests for it. We haven't had time to track down why.) We went to work and put *Ethyl Methanesulfonate* on the stockroom shelf as Eastman 7830. Then, as a switcheroo, but with a certain seriousness of purpose, we made *Methyl Ethanesulfonate* (Eastman 7876). Perhaps someone will buy it who wants a deeper understanding of why the interchange of methyl and ethyl groups between the ends of the molecule depresses but does not destroy biological activity.

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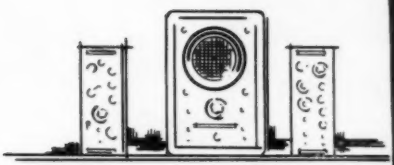

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CLINICAL AND EXPERIMENTAL

MYOCARDIAL BLOOD FLOW DETERMINED BY SURFACE COUNTING AND RATIO FORMULA

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OKLAHOMA CITY, OKLA.

A CONVENIENT method for the estimation of myocardial blood flow would be of considerable clinical importance. The methods now available, while useful in research, are not applicable to routine clinical testing since catheterization must be accomplished. We wish to report a method for estimating myocardial blood flow which circumvents this difficulty and which is applicable to routine clinical testing.

Huff and associates[1] and others have found that cardiac output can be estimated by following the passage of a radioactive tracer through the heart. This is accomplished by placing a gamma ray detector on the chest wall over the outflow tract of the heart and measuring the chronological change in counting rate. The curve produced during the first circulation of the radioactivity is described as having 2 well-defined peaks representing the passage of the radioactivity through the right and left sides of the heart, respectively. The area beneath these peaks is inversely proportional to the rate of blood flow through the heart. This value can be used in the dye dilution formula for cardiac output which was devised by Stewart, and revised by Kinsman and associates,[2] if the amount of radioactivity is converted to concentration by a calibration factor.

Attempting to reproduce the work of Huff and associates, we used an externally placed detector to measure cardiac output. Our time activity curves were found to include a third peak which closely followed the 2 peaks of the heart curve. The onset of this third peak coincided with the appearance of the

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radioactivity in the periphery. Since the major peripheral circulation seen by the detector is the heart itself, when the detector is over the projection of the heart, we felt that the area of this third peak might be related to myocardial blood flow. To test this idea we have compared the estimates of myocardial blood flow obtained by this method in a clinical survey and in various dog experiments.

MATERIALS AND METHODS

Equipment.—For detecting and recording, we used one heart channel and one carotid channel. Each channel consists of a scintillation detector, a rate meter, and a recorder. The scintillation detector has a 1½ by 2 inch sodium iodide crystal with a ⅛ inch lead filter enclosed in a cylindrical collimator. The crystal with its filter is flush with the top of the collimator. The output of this scintillation detector is fed into a linear rate meter (Tracerlab precision rate meter) with a 0.32 m.f.d. integrating condensor which allows the rate meter to go full scale in 0.3 second. Full scale represents 25,000 c.p.m. on the heart channel and 10,000 c.p.m. on the carotid channel. The rate meter is connected to a rectilinear recorder (Texas Instrument) recording at a speed of 12 inches per minute. The syringe used for injection of the tracer dose is a siliconized tuberculin syringe. The tracer substance used has been radioactive iodinated human albumin (R.I.S.A.) or Diodrast. The volume of the dose has been 0.2 to 0.4 ml. for humans and 0.05 to 0.1 ml. for dogs. The amount of radioactivity has been about 20 μc for humans and about 2 μc for dogs. The radioactivity can be corrected for machine sensitivity or vice versa, but we have found that the third peak is more easily seen with a small dose and a high sensitivity. It is important to have the peaks of the heart curve reach a height of about 20,000 counts per minute.

The extrapolation of the heart curve and myocardial blood flow peak are done mathematically by the method of least squares or by a French curve of best fit. The areas under each curve are determined by a planimeter.

The projection of the heart on the chest wall is found by a standard chest x-ray.

Procedure.—The patient is seated in front of the detectors. The projection of the heart is marked on the chest wall. The detector of the heart channel is placed over the middle point of the heart projection on the chest wall. The detector of the carotid channel is placed over a carotid artery. The purpose of the carotid channel is to register the start of the peripheral circulation and in this way identify the start of the third peak on the heart curve. These appear simultaneously on the recording because it moves at a rate considerably slower than that of arterial blood. A base line is obtained on the recording before the injection of the tracer dose.

The injection is made in the antecubital vein, preferably the basilic vein. The injection should be performed as rapidly as possible to prevent excessive dilution of the radioactive dye bolus. The curves are more distinct and easily interpreted when the dye bolus is small. The time of injection is marked by a 6 volt impulse released from a foot pedal. The exact amount of radioactivity in the syringe is determined by placing it in front of the detector before and after the injection. In this way, the actual percentage of the dose injected can be determined. Ten minutes later when complete mixing between blood and tracer has taken place a final recording is run with the patient in the same position and a blood sample is withdrawn. The concentration of radioactivity in this blood sample is determined in a well counter. A calibration factor for the system is obtained by calculating the ratio of the radioactivity of this blood sample and the reading on the recording made at the time the blood sample was taken. This factor also corrects counts per minute to concentration in the dye dilution formula.

Interpretation of the Curves.—Figs. 1 and 2 show typical curves obtained over the heart region and under each a curve obtained over the carotid artery. The tracing runs from right to left with the time as the base line. On the original curve 5 mm. represents 1 second. The height of the curve represents the count rate per minute as it appears under the

gamma ray detector. The point labeled injection is the time of injection of the radioiodinated albumin into the antecubital vein. At point H_1 on the heart curve the first radioactivity enters the right heart and, as more accumulates, the curve moves upward, reaches a maximum, and then begins to descend as the radioactivity passes into the pulmonary artery and the lungs. After traversing the pulmonary circuit, the radioactivity reappears in the left side of the heart and the curve again reaches a peak. This second peak lies on the downslope of the curve for the right heart as there is never any overlapping in a radioisotope

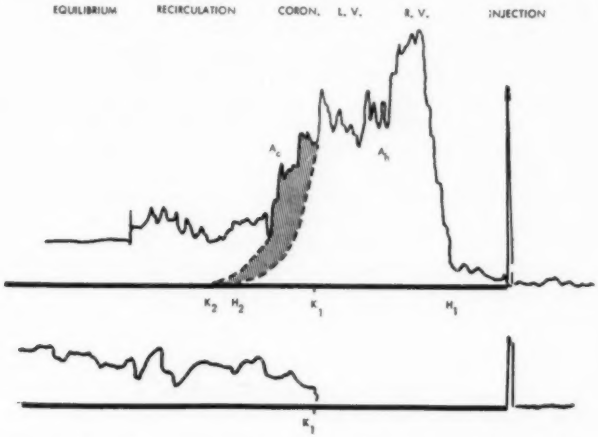

Fig. 1.—Tracings from a normal subject by body surface counting over the heart region, upper tracing, and the neck region, lower tracing, during the first circulation of the tracer. The shaded area represents the peripheral circulation in the heart region, the myocardial blood flow. Note that the peripheral circulation in heart and neck region starts simultaneously. Text contains explanations of letters.

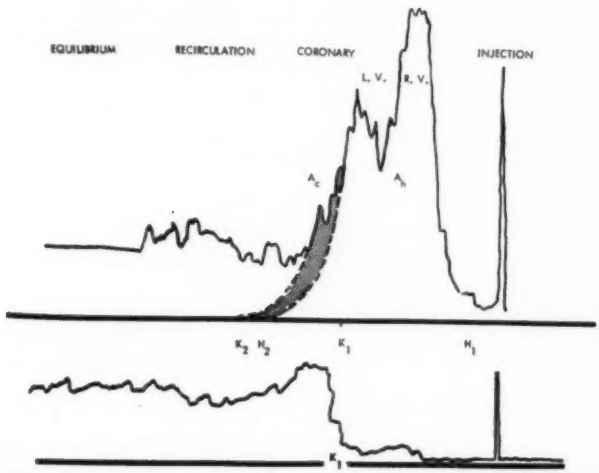

Fig. 2.—Tracings recorded over heart and neck regions in a patient with coronary occlusion. Note that the shaded area representing coronary circulation is smaller than that in Fig. 1.

tracing. At the time of the start of the peripheral circulation (point K_1) as it is recorded on the carotid channel, a third peak is recognized lying on the downslope of the peak from the left heart. The authors assume that this third peak is related to the peripheral circulation monitored in the heart region. The heart curve beyond the coronary peak and the coronary curve beyond recirculation have to be extrapolated to the base line (points H_2 and K_2, respectively). As in the case of the dye dilution curves, both are exponential beyond their deflection points and the exponential curve of best fit can be determined using the points lying between the deflection point and recirculation by the method of least squares. The authors used this method for one-half year. The technique was very time consuming with tiresome mathematical calculations; each curve took about 2 hours to construct. However, in going back over the mathematically constructed curves we found that we could do as well with a French curve of best fit (Keuffel and Esser Co. No. 1860-4), and that it was beyond the accuracy of the planimeter to detect the difference between the 2 methods. The heart curve, as it appears in Fig. 4 devoid of coronary circulation, fits the French curve almost exactly. Care must be exercised to include only the slope beyond the deflection point where the curve changes from convex to concave.

If ordinates are drawn upward from the extrapolated portion of the heart curve to the top of the coronary curve, it will be noted that at first the ordinates increase in height; this is followed by a decrease and then another increase. The point where these vertical heights increase for the second time is the moment when the first recirculation of the tracer begins. The coronary peak has to be extrapolated beyond this point and it is done in the same fashion as the extrapolation of the heart curve. The whole procedure is usually very easily accomplished.

The areas beneath both the heart and coronary curves are determined with a planimeter. The transit time for the radioactivity to pass the heart and the coronary is measured on the base line from the time where each curve starts to where each seems to end as they are extrapolated to the base line. Each 5 mm. represents 1 second (distances H_1-H_2 and K_1-K_2, respectively).

Mathematics.—Our aim is to determine cardiac output as Huff and associates did and then to determine the ratio of the cardiac output and the coronary blood flow during the first circulation of the radioactive tracer through each organ. By dividing the cardiac output by this ratio we can obtain the peripheral organ blood flow. Cardiac output is estimated by the method of Huff and co-workers, and we make use of the dye dilution formula of Kinsman and associates.

$$\text{Cardiac output} = \frac{I}{A \times k \times K}$$

$$I = \text{Injected dose} \frac{(\text{counts per minute [c.p.m.]} \times \text{milliliters injected})}{\text{milliliters}}$$

A = Area beneath the heart curve in square inches

k = Converts square inches on graph to c.p.m. × seconds

K = Calibration factor = ratio of c.p.m. per milliliter blood at equilibrium over c.p.m. recorded on the heart curve at the same time

In order to derive a formula for determination of the ratio between the cardiac output (F_h) and the coronary blood flow (F_c), the following formulation is first made. After the tracer dose (I) is injected into the blood stream it will be mixed homogenously with blood to form a tracer-blood bolus of size $\frac{I}{\int_o^a \Delta C}$ ml. The expression $\int_o^a \Delta C$ stands for the continuous change in concentration within the traced blood bolus as it mixes with the rest of the blood. As an approximate assumption the statement has to be made that the tracer dose within the blood bolus is homogenously mixed with the blood through the cross

section of the blood stream. The tracer will then behave and branch as the blood stream with which it mixes. The volume of the traced blood bolus (V) in each instance will be:

$$V = \frac{I}{\int_0^a \Delta C} \tag{1}$$

A certain amount of I represents a certain area (A) on the recording in direct proportion. According to the axiom, the area is proportional to one of its dimensions; in this case it is the height. The proportionality factor is the sensitivity (S) of the recording equipment. We can thus state:

$$V = \frac{I}{\int_0^a \Delta C} = \frac{A}{\int_0^a \Delta C \cdot S} \tag{2}$$

If this equation is expressed per time we receive an expression for flow (F):

$$F = \frac{V}{T} = \frac{I}{T \cdot \int_0^a \Delta C} = \frac{A}{T \cdot \int_0^a \Delta C \cdot S} \tag{3}$$

Specifically for heart (subscript h) and coronary (subscript c) this would be:

$$F_h = \frac{A_h}{T_h \cdot \int_0^a \Delta C_h \cdot S_h} \quad F_c = \frac{A_c}{T_c \cdot \int_0^a \Delta C_c \cdot S_c} \tag{4}$$

Another expression for organ blood flow is obtained from the dye dilution formula for each organ, again making use of the detected traced total blood volume instead of just a tracer substance as the tracer. The concentration between the tracer substance within the blood bolus is not known as a continuous dilution is occurring. Let the integral $\int_0^a \Delta C_h$ at the heart level and $\int_0^0 \Delta C_c$ at the coronary level express this changing concentration. We can then express cardiac output and coronary blood flow as:

$$F_h = \frac{I_h \cdot \frac{1}{\int_0^a \Delta C_h} \cdot S_h}{A_h} \quad F_c = \frac{I_c \cdot \frac{1}{\int_0^a \Delta C_c} \cdot S_c}{A_c} \tag{5}$$

If formulas (4) are substituted into formulas (5), one receives:

$$\frac{A_h}{T_h \cdot \int_0^a \Delta C_h \cdot S_h} = \frac{I_h \cdot S_h}{A_h \cdot \int_0^a \Delta C_h} \tag{6}$$

and

$$\frac{A_c}{T_c \cdot \int_0^a \Delta C_c \cdot S_c} = \frac{I_c \cdot S_h}{A_c \cdot \int_0^a \Delta C_c} \tag{7}$$

As the concentration integrals within each equation are the same, these cancel out. With each equation solved for I, the ratio between the injected doses into each organ is then determined; one receives:

$$\frac{I_h}{I_c} = \frac{A_h^2 \cdot S_c^2 \cdot T_c}{A_c^2 \cdot S_h^2 \cdot T_h} \tag{8}$$

The heart flow and the coronary blood flow are both recorded in the same channel so that in this particular instance $S_h = S_c$ and cancel each other. The amount of tracer entering each organ is proportional to that organ flow if, as we assumed from the very beginning, complete

mixing between blood and tracer is accomplished in the cross section of the blood vessel.[5] We consider this assumption reasonable and then finally state coronary blood flow as:

$$F_c = F_h \div \frac{A_h^2 \cdot T_c}{A_c^2 \cdot T_h} \tag{9}$$

Model Circulation.—To prove the above mathematically derived ratio formula for branched flow we attempted the following experiment in a flow model. The arrangement was the same as in Fig. 3. Water was siphoned from a large tank through a principal tube down to a bifurcation where the flow was divided into 2 branches, each one emptying into a container. Cylinders were inserted into the principal tube and into one branch. This simulated the heart with the unbranched flow and the coronary system with the branched flow. Each cylinder was filled with glass beads to achieve turbulent flow. The whole system was filled with water. The water flow was siphoned through all tubings at a constant flow rate. The water from each branch was measured per unit time. The sum of each branch flow was the total "heart flow." The heart cylinder (labeled A) and the coronary cylinder

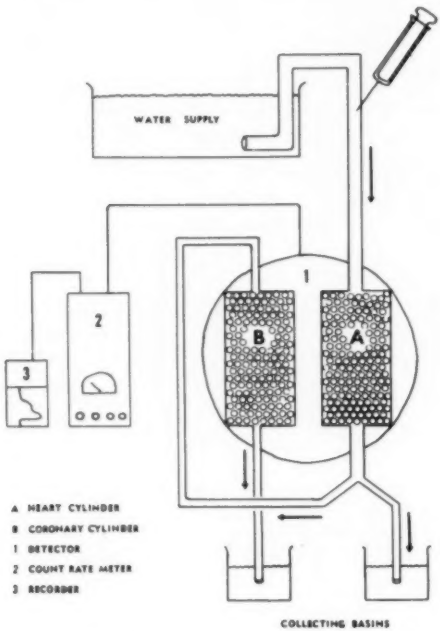

Fig. 3.—Diagram of flow model.

(labeled B) were placed in front of the detector. The machine was arranged as in the clinical work. A tracer dose of a few μc, dissolved in 0.05 ml. of saline, was injected above the heart cylinder into the principal flow. As the tracer dose passed through the heart cylinder, a flow curve for total flow was registered on the recorder. Below the heart cylinder, the flow was divided and part of it repassed the detector in the coronary cylinder. The total flow and branched flow were determined by the measurement of the water in each container. The ratio between total flow and coronary blood flow was calculated from the resulting data. Our aim was to determine whether or not this ratio could also be obtained by monitoring over the cylinders and calculating the ratio from the registered flow curves.

A typical flow curve from the experiments is reproduced in Fig. 4. The results are shown in Table I. The correlation coefficient between the exact measured flow and our estimated flow is 0.933. This proves that the ratio formula allows one to determine flow by an external monitoring technique.

TABLE I. COMPARISON IN A FLOW MODEL BETWEEN DETERMINATION OF BRANCHED FLOW BY DIRECT MEASURING AND BY SURFACE COUNTING

H_2O FLOW ML. PER GIVEN TIME		RATIO	AREA IN SQUARE INCHES		TIME IN SECONDS		RATIO
F_h	F_c	$\frac{F_h}{F_c}$	A_h	A_c	T_h	T_c	$\frac{F_h}{F_c}$
249	79	3.15	3.0	1.8	14.0	16.6	3.29
370	172	2.15	3.2	2.9	12.8	22.0	2.09
582	220	2.65	4.3	4.4	17.6	48.0	2.60
546	206	2.65	5.4	3.8	21.8	29.0	2.69
604	264	2.29	5.2	4.4	20.0	31.6	2.21
598	366	1.63	5.5	5.0	23.0	31.2	1.64
348	190	1.83	5.6	5.0	23.6	32.8	1.74
533	185	2.88	4.2	4.4	17.8	45.0	2.30
312	136	2.34	2.9	3.1	13.0	35.0	2.35*
226	76	2.97	2.6	3.1	11.0	46.0	2.94
266	102	2.61	4.2	3.8	11.3	24.1	2.63
198	90	2.20	5.2	4.3	15.7	23.3	2.17
293	103	2.84	4.9	4.2	15.5	31.8	2.79

*Refers to Fig. 4.

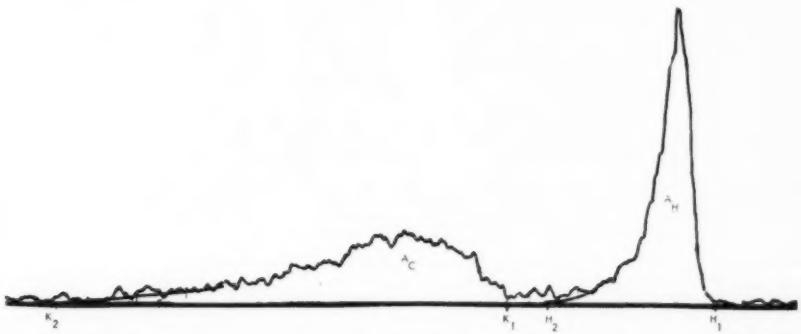

Fig. 4.—Typical time dilution curve recorded by surface counting technique in the flow model. The first (A_H) represents unbranched flow. The following lower peak represents the branched flow (A_C).

RESULTS

Dog Experiments.—Fig. 5 shows 2 tracings recorded at a dog experiment. The chest was open and the aorta was in normal position behind the heart. The scintillation tube was placed over the chest incision at a chest-wall distance from the heart. Both coronary vessels were dissected free and a loop of string was placed around them. The dose of radioactivity was 2 μc in a 0.07 ml. volume. The upper recording was done first with the coronary vessels open. The typical curve was obtained. The lower curve was recorded shortly after with both coronary vessels occluded. On this recording the coronary peak has disappeared completely. A space is noted between the heart curve and the appearance of recirculation. This shows that radioactivity is not recorded from the descending

aorta. It also demonstrates that the third peak represents coronary blood flow. This experiment was accomplished with the surgical assistance of Dr. David Snyder.

To investigate the accuracy of this method, coronary blood flow was determined by simultaneously using both the nitrous oxide method[3] and this radioisotope technique. These experiments were done at Washington University, St. Louis, with the help of Drs. R. Bing, W. Danforth, and F. Ballard, who performed the nitrous oxide determinations. Because of the difficulties involved, only 1 human experiment was done. The radioisotope technique gave a 17 per

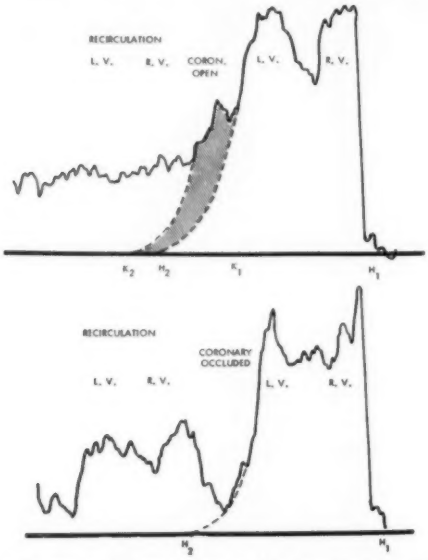

Fig. 5.—Time dilution curves recorded from a dog experiment. The upper tracing is recorded with the coronary vessels open, the lower tracing when the coronary vessels are temporarily occluded. Note the disappearance of the area representing the coronary blood flow.

cent higher estimate than did the N_2O method using a nomogram for heart weight. In dog experiments where the heart weight can be measured, the N_2O method gives a more exact estimate of total coronary blood flow. The first 4 experiments were done with the chest wall of the dog closed. The values of the radioisotope technique were about 17 per cent greater than the N_2O values. No significant difference is noted when the chest wall was opened or when the aorta was displaced out of range of the detector. A very close statistical correlation is also noted between the two techniques ($r = + 0.967$). However, this does not prove that the 2 methods are exactly the same, but it does show that they follow each other very closely through a wide range of values. The results are listed in Table II along with cardiac output determinations using the method of Fick and the method of Huff.

Clinical Study.—Fig. 6 shows the results from a clinical study of myocardial blood flow in 47 patients without and 18 patients with known coronary

TABLE II. CARDIAC OUTPUT (F_h) DETERMINED WITH I^{131} AND CO_2 AND CORONARY BLOOD FLOW (F_c) DETERMINED WITH I^{131} AND N_2O SIMULTANEOUSLY

	F_h I PER MINUTE		F_c MILLILITER PER MINUTE		
	CO_2	I^{131}	N_2O	I^{131}	SUBJECTS
1	6.79	6.73	254	305	30-year-old patient
2	2.69	3.17	246	302	Dog chest closed
3	4.91	4.93	258	238	Dog chest closed
4	1.53		51	65	Dog chest closed
5	2.33	2.32	144	194	Dog chest closed
6		1.01	53	63	Dog open chest
7	0.60	0.67	42	49	Dog open chest Displaced aorta

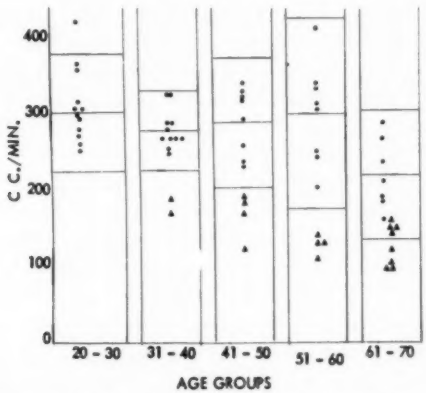

Fig. 6.—Coronary blood flow values in different age groups. Patients without known coronary disease are marked as solid dots. Patients with known coronary disease are marked as triangles. Mean and 2 S.D. are marked in each age group as solid lines. Note the overlapping between normal individuals and diseased patients in the seventh decade.

disease using this radioisotope technique. These patients were matched for size and weight. The patients with coronary disease were ambulatory patients most of whom were recovering from myocardial infarctions. The determinations were done in a resting state with the patient seated. The data has been divided according to decades. The diagnosis of coronary occlusion was confirmed by electrocardiogram, history, and physical examination. It is noted that in the resting state coronary blood flow remains about the same up to an age of 60 years. A smaller difference is noted between the normal range in each decade and the infaret values as the patient grows older; these actually overlap in the oldest age group. The mean of the total normal group is 284 ml. per minute and the normal range is from 184 to 384 ml. per minute (mean ± 2 standard deviations).

DISCUSSION

By now, many reports have been published on the usefulness of the surface counting technique for determining cardiac output. The authors usually describe the heart curve as consisting of 2 peaks, 1 for each side of the heart. We have found that the typical curve is composed of 3 peaks with the third peak

produced by myocardial blood flow. There are several reasons why others have failed to find this third peak. Because of the irregularity of these tracings, others have purposely smoothed out these curves as much as possible in order to make the extrapolation simpler. This has been accomplished by longer time response in the rate meters, lower sensitivity settings, and faster recording speeds, all of which tend to obscure the relatively small third peak. Some investigators have made use of narrow collimation which excludes most of the coronary circulation. Still others have excluded coronary blood flow by placing the detector above the heart over the second intercostal space. In a few publications, however, the third peak is easily recognized as a convexity on the downslope of the second peak.[4]

The duration of the second peak represents 3 or 4 heartbeats. On first thought it might seem that the coronary peak would appear immediately after the start of the second peak and not on its downslope. This would be true if this peak represented only the radioactivity in the left ventricle. However, the second peak is composed of more than this since radioactivity is recorded as it approaches, passes through, and leaves the left ventricle. The simultaneous appearance of the peripheral circulation in the neck region and the start of the third peak is a good proof that the third peak is produced by the peripheral circulation in the heart region. The extremely short time required for arterial blood to pass from the heart to the carotid artery would require a very fast recorder to demonstrate a time difference between these 2 peaks.

The blood leaving the left heart is divided between the different organs in proportion to each organ's blood flow. If a tracer is added to blood and complete mixing is achieved from wall to wall, the tracer would be divided according to the blood flow. If the portion of the traced blood entering each organ can be determined, each organ's blood flow could be estimated. We have recognized the myocardial blood flow on tracings recorded over the heart. When organ blood flow is obtained on a tracing, we realize that many different techniques can be used to estimate quantitatively or qualitatively the blood flow to that particular organ. Mathematically we have derived a formula that gives values for flow which agree with estimates obtained by other techniques. The formula has been verified using a flow model. In a clinical survey this formula gives values that appear physiologic and which separate normal from pathologic blood flow. We have also used the same formula in pilot studies for estimation of blood flow in kidneys, uterus, and brain. In all, the values obtained appear physiologic.

Two extrapolations are required in this technique. The coronary peak lies on the extrapolation of the second heart peak. It is obvious that this introduces inaccuracies in our estimates of coronary blood flow. However, in duplicate testing and having different individuals perform these extrapolations, we find that values vary only ± 15 per cent. Because the transit time is divided into the square of the area, any change in area produced by mistakes in extrapolation is partially compensated for by the ratio formula. Therefore, we do not

expect the values obtained to be exact measurements but they do appear to be good estimates. Because the method is so fast and simple we feel it will be of value in clinical work.

SUMMARY

A new method for determination of peripheral organ blood flow by a surface counting technique has been presented. The technical design, the graphical procedures, and the mathematical handling have been described. The method has been used for determination of coronary blood flow and the values so obtained compare favorably with those obtained by a classical method. In a clinical study, the new technique has proved to be extremely simple, repeatable, rapid, and nontraumatic and can in this sense be compared to a simple x-ray procedure.

REFERENCES

1. Huff, R. L., Feller, D. D., Judd, O. J., and Bogardus, G. M.: Cardiac Output of Men and Dogs Measured by In Vivo Analysis of Iodinated (I^{131}) Human Serum Albumin, Circulation Res. **3**: 564, 1955.
2. Kinsman, J. M., Moore, J. W., Hamilton, W. F., and Spurling, R. G.: Studies on the Circulation. I and II, Am. J. Physiol. **89**: 322, 1929.
3. Bing, R. J., Hammond, M. M., Handelsman, J. C., Powers, S. R., Spencer, F. C., Eckenhoff, J. E., Goodale, W. T., Hofkenschiel, J. H., and Kety, S. S.: The Measurement of Coronary Blood Flow, Oxygen Consumption and Efficiency of the Left Ventricle in Man, Am. Heart J. **38**: 1, 1949.
4. Shackman, R.: Radioactive Isotope Measurements of Cadiac Output, Clin. Sc. **17**: 317, 1958.
5. Van der Feer, J.: The Determination of Cardiac Output by the Injection Method, Proefschrift, Excelsior, Oranjeplein. 96 S-Gravenhage.

APPENDIX

As an example the calculations for Figs. 2 and 3 were as follows. The values are arranged in the following order:

$$F_h = \frac{\text{Standard cts.} \times \text{dilution factor} \times \text{ml. inj.} \times \% \text{ inj.} \times \text{equil. reading}}{A_h \times k \times \text{bld. at equil.} \times 1000}$$

$$\text{Ratio} = \frac{A_h^2 \times T_c}{A_c^2 \cdot T_h}$$

$$F_c = \frac{F_h}{\text{Ratio}}$$

Calculation of F_c for Fig. 1:

$$F_h = \frac{424622 \times 1000 \times 0.4 \times 0.9 \times 1400}{6.4 \times 185 \times 34280 \times 1000} = 5.3 \text{ L. per minute.}$$

$$\text{Ratio} = \frac{6.4^2 \times 9}{1^2 \times 17.4} = 21.2$$

$$F_c = \frac{5.3}{21.2} = 0.243 \text{ L. per minute.}$$

Calculations of F_c for Fig. 2:

$$F_h = \frac{466948 \times 1000 \times 0.35 \times 0.95 \times 2100}{6.8 \times 185 \times 43137 \times 1000} = 6.0 \text{ L. per minute.}$$

$$\text{Ratio} = \frac{6.8^2 \times 8.8}{0.7^2 \times 17.8} = 46.7$$

$$F_c = \frac{6.0}{46.7} = 0.128 \text{ L. per minute.}$$

VASCULAR ANOMALY ASSOCIATED WITH PLASMA THROMBOPLASTIN ANTECEDENT DEFICIENCY

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HEMORRHAGIC syndromes with a prolonged bleeding time associated with a disturbance of the mechanism of coagulation have been reported by various authors.[1-10] Thus far the prolonged bleeding time has been found in association with deficiencies of antihemophilic globulin (AHG, factor VIII) and plasma thromboplastin component (PTC, factor IX). The cause of the prolonged bleeding time is not uniform. In certain cases capillary microscopy disclosed definite changes,[1, 4] in others there appears to be a deficiency of a plasma factor which normally assures capillary integrity,[8] and in others there may be a functional platelet defect.[10]

This is a report of a patient with vascular purpura and plasma thromboplastin antecedent (PTA) deficiency.

MATERIALS AND METHODS

Clotting Time of Venous Blood.—The tests are carried out in duplicate at room temperature (25° C.) in siliconized or plain glass tubes with an internal diameter of 12 mm. Two milliliters of blood was placed in each tube.

Plasma Recalcification Time.—The time interval between the addition of 0.1 ml. of 0.025 M $CaCl_2$ to 0.1 ml. of plasma at 37° C. and the appearance of a clot was determined.

Test Methods.—Bleeding time was determined by the method of Duke.[11] For the capillary fragility test, the method of Rumpel-Leede[12] was used. Determination of platelet count was made by phase microscopy according to Feissly and Lüdin.[13] Quick's[14] one-stage prothrombin time was used with human brain thromboplastin. Prothrombin, prothrombin consumption, and factor VII complex were determined according to Koller and associates.[15] Factor V was determined by the method of Kappeler,[16] fibrinogen with the technique of Clauss,[17] factor VIII and factor IX using the method of Geiger and co-workers,[18] and the Stuart-Prower factor according to the method of Bachmann and associates.[19] The thromboplastin generation test was performed with the modified technique of Duckert and co-workers.[20]

One-Stage Assay for PTA.—The plasmas of two different patients with congenital PTA deficiency[21] were used as substrates. The technique was as follows: 0.1 ml. of PTA-deficient substrate plasma, 0.1 ml. of normal plasma diluted 1:10 with 0.9 per cent NaCl solution, and 0.1 ml. of chloroform brain extract in optimal concentration (1:100) were incubated at 37° C. for 30 seconds. Then, 0.1 ml. of 0.025 M $CaCl_2$ was added and the clotting time recorded. The results obtained with several normal plasmas were averaged and represent the 100 per cent value. There is a wide individual range of the normal values corresponding to a concentration of 40 to 250 per cent of the average. A standard reference curve was obtained by further dilutions of normal plasma (Fig. 2). The PTA activity of an unknown plasma was determined by substituting the normal control plasma with the unknown.

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CASE REPORT

T. C., a 21-year-old white woman, underwent an appendectomy at the age of 9 years without hemorrhagic complications. Menarche occurred at the age of 13 years. There was no history of menorrhagia or metrorrhagia. At the age of 15 years she began having bouts of purpura over the lower extremities and arms. There were no specific precipitating factors, in particular there was no relation of the purpura to the menstrual cycle. The patient did not bleed excessively from minor cuts or abrasions, but she bruised rather easily. She was treated with vitamin C, rutin, ACTH, and cortisone without success. The family history was negative for hemorrhagic diseases; her grandparents, parents, two brothers, and one sister had had no hemorrhagic symptoms.

On physical examination there was a morbilliform, partly confluent brown pigmentation of the lower extremities and the forearms as a result of the long-standing purpura. There were also fresh petechiae. As an incidental finding the patient had a beginning detachment of the retina in the right eye. Surgery was performed after the transfusion of 450 ml. of fresh blood. There was no unusual bleeding and the operative result was excellent.

Laboratory Data.—The urinalysis was negative. Hemoglobin was 13.7 Gm. per cent, and leukocytes 5,700 with a normal differential count. The total protein was 7.1 Gm. per cent with a normal electrophoretic distribution of 61 per cent albumin, 9 per cent α-globulins, 12 per cent β-globulins, and 18 per cent γ-globulins. The cryoglobulins were negative. Cytologic studies of vaginal smears over a whole menstrual cycle revealed no abnormalities. However, the microscopic picture of the nail bed capillaries revealed striking abnormalities. The capillaries were extremely tortuous with a marked variability in caliber and an occasional anastomosis. Normal hairpin-shaped loops could be found only rarely.

RESULTS

The tests of hemostasis are summarized in Table I. The patient had a strongly positive Rumpel-Leede test, a prolonged bleeding time, and a decreased prothrombin consumption. The thromboplastin generation test is

TABLE I. TESTS OF HEMOSTASIS

TEST	PATIENT T. C.	NORMAL VALUES
Whole blood clotting time in silicone	75-85 min.	25-70 min.
Whole blood clotting time in glass	9½-21 min.	6-22 min.
Plasma recalcification time	75-120 sec.	60-120 sec.
Prothrombin consumption test (6 hr.)		
Residual prothrombin in serum	11-17%	1-4%
Bleeding time	3-11 min.	1-3 min.
Rumpel-Leede test	Strongly positive	Negative
Clot retraction	Normal	
Platelet count/c.mm.	158-267,000	150-400,000
Platelet morphology	Normal	
One-stage prothrombin-time (Quick)	80-100%	70-100%
Fibrinogen Prothrombin Factor V Factor VII Stuart-Prower II factor	Normal	
Factor VIII (AHG), one-stage method	100%	50-150%
Fibrinolysis	Negative	
Antithrombin	Normal	

illustrated in Fig. 1. The most abnormal results were obtained when the patient's plasma and serum were used jointly. The thromboplastin generation test with the patient's serum alone gave variable results; at times it was slightly pathologic, on another occasion it fell within normal limits.

The thromboplastin generation test with the patient's plasma was always normal. These findings were strongly suggestive of a deficiency of PTA. This assumption was confirmed by mixing experiments with blood from known cases of PTA deficiency (Table II). The undiluted patient's plasma was less effective than undiluted normal plasma and even 1:5 dilution of normal plasma in buffer. The patient's PTA activity, therefore, must have been less than 20 per cent of normal. This was in agreement with the results obtained

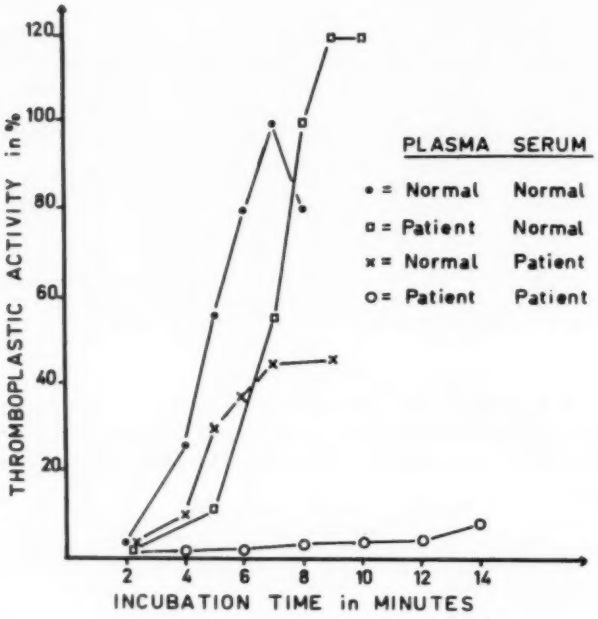

Fig. 1.—Thromboplastin generation test.

TABLE II. EFFECT OF PATIENT T. C.'S PLASMA VERSUS NORMAL PLASMA ON THE CLOTTING TIME AND PROTHROMBIN CONSUMPTION OF PTA-DEFICIENT BLOOD

TO 2.0 ML. OF PTA-DEFICIENT BLOOD WERE ADDED 0.2 ML. OF:	CLOTTING TIME IN MINUTES	RESIDUAL PROTHROMBIN ACTIVITY IN % (24 HR.)
Michaelis buffer	48	82
Plasma of Patient T. C. undiluted	19.30	17
Plasma of Patient T. C. diluted 1:5 in buffer	24.30	29
Normal plasma, diluted 1:5 in buffer	14	2.2

All determinations were done in duplicate. The clotting times were determined at room temperature (25° C.). After clotting, the mixtures were incubated in a water bath at 37° C. for 4 hours, and stored another 20 hours at room temperature before determination of the residual prothrombin in the sera.

with the more quantitative assay described under Materials and Methods (Fig. 2). The patient's plasma was tested with two different PTA-deficient substrates and was found to have a PTA activity of 11 and 12 per cent, respectively, of normal. In order to eliminate the possibility of a deficiency

of PTC (factor IX) which could account for the inconstantly abnormal thromboplastin generation test when the patient's serum was used alone, mixing experiments were performed with PTC-deficient plasma. The results in Table III clearly show that PTC-deficient plasma was at least as effective as normal plasma in correcting our patient's abnormal prothrombin consumption. This excludes a deficiency of plasma thromboplastin component in our case.

TABLE III. EFFECT OF PTC-DEFICIENT PLASMA VERSUS NORMAL PLASMA ON THE CLOTTING TIME AND PROTHROMBIN CONSUMPTION OF PATIENT T. C.'S BLOOD

TO 2.0 ML. OF PATIENT T. C.'S BLOOD WERE ADDED 0.2 ML. OF:	CLOTTING TIME IN MINUTES	RESIDUAL PROTHROMBIN ACTIVITY IN % (24 HR.)
Michaelis buffer	21	16
Normal plasma, diluted 1:10 with buffer	20	6
PTC-deficient plasma, diluted 1:10 with buffer	12.30	3.5

Technique as in Table II.

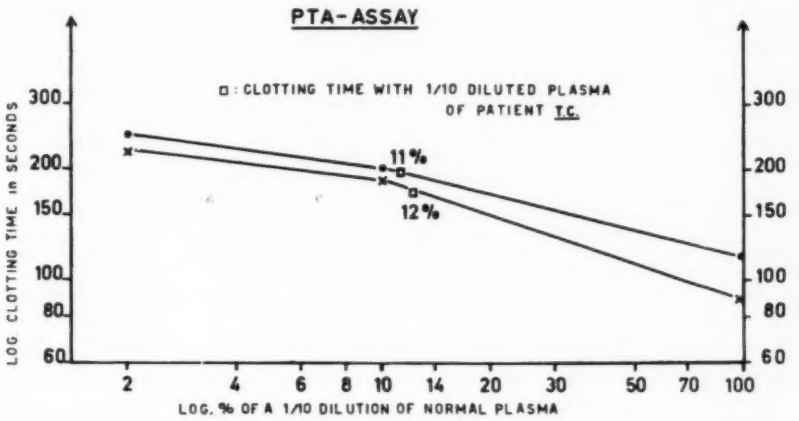

Fig. 2.—Plasma PTA activity of Patient T. C. Key: ×—× and •—• = standard reference curves with plasmas of two individuals with congenital PTA deficiency; □ = clotting times and corresponding PTA activity of Patient T. C.'s plasma. Technique as described under Materials and Methods.

DISCUSSION

The simultaneous occurrence of a vascular defect with a deficiency of PTA adds a new entity to the large list of hemorrhagic diseases. The present case differs from the majority of other defects of coagulation associated with a prolonged bleeding time in two respects: (1) there is no proof of heredity, and (2) the purpura became manifest only at the age of 15 years. The negative family history over 3 generations is unusual if one compares our case with the hereditary character of the prolonged bleeding time and the simultaneous deficiency of AHG and PTC reported in other patients.[3, 6-8]

The onset of purpura after puberty remains unexplained. One is tempted to relate the purpura to the hormonal changes occurring at this age. However,

this is mere speculation since the purpura was not related to the menstrual periods. It will be of interest to know if the bouts of purpura subside with menopause.

SUMMARY

A report has been presented of a 21-year-old woman with severe purpura secondary to abnormal capillaries and plasma thromboplastin antecedent (PTA) deficiency.

REFERENCES

1. Alexander, B., and Goldstein, R.: Dual Hemostatic Defect in Pseudo-Hemophilia, J. Clin. Invest. (Abst.) **32:** 551, 1953.
2. van Crefeld, S., Jordan, F. L. J., Punt, K., and Veder, H. A.: Deficiency of Antihemophilic Factor in a Woman, Combined With a Disturbance in Vascular Function, Acta med. scandinav. **151:** 381-389, 1955.
3. Schulman, I., Smith, C. H., Erlandson, M., Fort, E., and Lee, R. E.: Vascular Hemophilia. A Familial Hemorrhagic Disease in Males and Females Characterized by Combined Antihemophilic Globulin Deficiency and Vascular Abnormality, Pediatrics **18:** 347-361, 1956.
4. Matter, M., Newcomb, R. F., Melly, A., and Finch, C. A.: Vascular Hemophilia: The Association of a Vascular Defect With a Deficiency of Antihemophilic Globulin, Am. J. M. Sc. **232:** 421-433, 1956.
5. Koch, F., Schultze, H. E., Schwick, G., Klees, E., and Kuntze, E.: Zur Problematik der Thrombopathia Willebrand-Jürgens, der Pseudohämophilie bzw. weiblichen Hämophilie, Ztschr. Kinderh. **79:** 449-464, 1957.
6. Klesper, R., and Achenbach, W.: Über eine neue Familie mit Angiohämophilie A (vaskuläre Hämophilie), Thromb. Diath. hämorrh. **1:** 223-233, 1957.
7. Bernard, J., Larrieu, M. J., Caen, J., and Maroteaux, P.: Maladies hémorrhagiques familiales associant un allongement du temps de saignement à un déficit en facteur prothromboplastique plasmatique, Haemotologica **42:** 1231-1250, 1957.
8. Nilsson, I. M., Blomback, M., and von Francken, I.: On an Inherited Autosomal Hemorrhagic Diathesis With Antihemophilic Globulin (AHG) Deficiency and Prolonged Bleeding Time, Acta med. scandinav. **159:** 35-57, 1957.
9. Soulier, J. P., and Larrieu, M. J.: Déficit en facteur antihémophilique B avec allongement du temps de saignement, Sang **28:** 138-144, 1957.
10. Jürgens, J., and Jürgens, R.: Untersuchungen zur Pathogenese der Thrombopathien, Proceedings of the International Congress of Hematology, Rome, 1958, pp. 259-260.
11. Duke, W. W.: The Relation of Blood Platelets to Hemorrhagic Disease, J. A. M. A. **55:** 1185-1192, 1910.
12. Wintrobe, M. M.: Clinical Hematology, ed. 3, Philadelphia, 1952, Lea & Febiger, p. 263.
13. Feissly, R., and Lüdin, H.: Microscopie par contrastes de phases. III. Applications à l'hématologie, Rev. hémat. **4:** 481-501, 1949.
14. Quick, A. J., Stanley-Brown, M., and Bancroft, F. W.: A Study of the Coagulation Defect in Hemophilia and Jaundice, Am. J. M. Sc. **190:** 501-511, 1935.
15. Koller, F., Loeliger, A., and Duckert, F.: Experiments on a New Clotting Factor, Acta haemat. **6:** 1-16, 1951.
16. Kappeler, R.: Das Verhalten von Faktor V im Serum unter normalen und pathologischen Bedingungen, Ztschr. klin. med. **153:** 103-113, 1955.
17. Clauss, A.: Gerinnungsphysiologische Schnellmethode zur Bestimmung des Fibrinogens, Acta haemat. **17:** 237-246, 1957.
18. Geiger, M., Duckert, F., and Koller, F.: Quantitative Bestimmungen von Faktor VIII (antihämophiles Globulin) und Faktor IX (Christmas Factor, PTC) bei Blutersippen, Fifth Congress of the European Society of Hematology, Heidelberg, 1956, Springer-Verlag, pp. 413-417.
19. Bachmann, F., Duckert, F., and Koller, F.: The Stuart-Prower-Factor Assay and Its Clinical Significance, Thromb. Diath. haemorrh. **2:** 24-38, 1958.
20. Duckert, F., Flückiger, P., Isenschmid, H., Matter, M., Vogel-Meng, J., and Koller, F.: A Modification of the Thromboplastin Generation Test, Acta haemat. **12:** 197-202, 1954.
21. Bachmann, F., Duckert, F., Fisch, U., Streuli, F., Gerber, D., and Koller, F.: Der Gerinnungsdefekt beim kongenitalen PTA-Mangel, Schweiz. med. Wchnschr. **88:** 1037-1044, 1958.

PROTEIN FRACTIONATION

I. Chromatography of Human Serum Proteins on DEAE-Cellulose With Special Reference to Blood Group Antibodies

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PETERSON and Sober and their co-workers[1, 2] have reported the preparation of modified celluloses and their use for ion exchange chromatography of proteins. Their studies, which have primarily utilized the 2-diethylaminoethyl ether derivative (DEAE-cellulose) as an anion exchanger, have been directed toward developing a general system for resolving complex protein mixtures. Among the earlier reports dealing with specific applications of this technique is that of Mitz and Yanari,[3] who achieved two- to tenfold increases in the purity of kidney cathepsin and liver catalase. Ellis and Simpson[4] reported the purification of growth hormone by chromatography on DEAE-cellulose, and Lewis and Thiele[5] fractionated pancreatic insulinase on this adsorbent. Humphrey and Porter[6] have studied chromatographically the reagin content of sera from hyperallergic individuals, and more recently Fahey, McCoy, and Goulian[7] have reported extensive studies with a variety of human serum samples. As a step toward evolving a fractionation scheme of wide applicability, the present paper is concerned with the effect of several eluting systems on resolution of human serum proteins chromatographed on DEAE-cellulose. Chromatograms have been evaluated by optical density measurements, determination of electrophoretic mobilities, distribution of selected radioactively labeled proteins, and determination of the distribution of blood group antibodies.

MATERIALS AND METHODS

Serum Samples.—Serum samples, removed from clotted whole blood collected without anticoagulant, were stored frozen until used. Serum Tu was obtained from a group B, Rh positive donor whose anti-A titer increased markedly after receiving injections of Witebsky substance (Sharpe & Dohme group-specific Substances A and B). Anti-A titered 1: 2,000 in saline and 1: 16,000 in the indirect Coombs titration. Serum from Patient Jo was obtained from a group O, Rh negative donor who developed anti-D after receiving multiple transfusions during World War II. He has since received periodic injections of Rh positive cells to stimulate production of Rh antibody. With A,B, Rh negative cells, this serum gave saline and indirect Coombs titers of 1: 512 and 1: 4,000, respectively, and zero and 1: 4,000, respectively, with group O, Rh positive cells.

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Preparation of Adsorbent.—DEAE-cellulose (Brown Company, Type 20), a weak base anion exchanger was used throughout the study. It was prepared for use by first sieving it through a 100 mesh screen and collecting it on a 300 mesh screen. This material was then alternately washed 2 to 3 times by decantation with 0.1 N NaOH and 0.1 N H_3PO_4. The hydroxide form was brought to the pH of buffer I by suspending it in either glycine or H_3PO_4, as the case might be, at the concentration at which they were present in buffer I. Equilibration was completed by washing with buffer I itself.

The absorbent (approximately 20 Gm.) was poured as a slurry into a 22 mm. diameter chromatographic column. To achieve a flow rate of 15 to 20 ml. per hour, it was packed under 15 to 20 pounds per square inch N_2 pressure to a height of about 30 cm. After completion of a chromatogram, the adsorbent was removed to a large beaker and was regenerated by washing by decantation successively with 0.1 N NaOH, water, and 0.1 N H_3PO_4. Its exchange capacity of 0.38 mEq. per gram was essentially unchanged after use in 12 successive experiments.

Development of the Chromatogram.—After thorough dialysis against buffer I, the serum was added to the column in 5 ml. aliquots. Buffers (indicated by Roman numerals in Figs. 2, 3, and 4) were added dropwise from either a tight-fitting separatory funnel or an inverted bottle with a tube just below the liquid level in a 500 ml. Wolff bottle, which served as a mixing chamber. The latter, which was attached directly to the column, was agitated by a magnetic stirrer. The inverted bottle technique should not be used if there is appreciable difference in density between the solutions in the upper reservoir and mixing chamber. Gradients of increasing ionic strength and decreasing pH were used. Fractions were collected for the most part at hourly intervals using automatic fraction collectors. All operations were performed in a cold room at 5° C.

Optical Density and pH.—Optical density measurements were made on each fraction at 280 mμ using the Beckman DU spectrophotometer. pH was measured on random fractions with a Beckman Model M pH meter.

Electrophoresis.—Combined fractions (indicated by capital letters in the Figs. 2, 3, and 4) were dialyzed against distilled water at 5° C. and lyophilized. The dried protein was redissolved in 0.9 per cent NaCl, and electrophoresis was conducted on paper using veronal buffer at pH 8.6. Individual fractions of chromatogram 1 were examined electrophoretically. Staining was with a bromphenol blue-mercuric chloride mixture. Electrophoretic analysis was performed simultaneously on a normal serum sample, and each protein component of the fraction was classified by comparison to the serum component with which it corresponded most closely.

Serology.—Several different serologic tests were performed, in some instances on the individual fractions, in others, on the combined fractions. The saline agglutination test was done by adding to one drop of fraction, a drop of 2 per cent saline suspension of human erythrocytes. The tubes were incubated for 30 minutes at room temperature and then read microscopically. The albumin test was similarly conducted except that the cells were suspended in 20 per cent albumin. The indirect Coombs test was performed by centrifuging the saline test and removing the supernatant. The cells were washed 3 times with 0.9 per cent NaCl and a drop of Coombs serum (goat) was added; the mixture was incubated for 5 minutes at 37° C., centrifuged, and read microscopically.

Radioactivity.—Radioactivity was measured on each fraction of chromatogram 1 with a Nuclear Instrument scintillation well counter, No. DS-3, and scaler, No. 183. Since both I^{131} and Fe^{59} are beta and gamma emitters, a differential analysis of their relative contributions to the total radiation intensity was necessary. At the normal operating voltage of the well counter, 1,200 volts, the total activity was measured; at 750 volts only Fe^{59} activity was measured. From the ratio of Fe^{59} activity at 1,200 and 750 volts, the Fe^{59} contribution to total activity (1,200 volts) was calculated. I^{131} activity was determined by

difference. For each fraction the results are expressed as a percentage of the original respective isotope. Decay corrections and instrument variance were eliminated by measuring the total activity of an aliquot of the chromatogram charge with each day's assay.

RESULTS

Serum Tu was selected for study because its high titer of anti-A provided a good opportunity for following this biologic activity as the chromatogram developed. As a further aid to evaluation of fractionation, tracer quantities of transferrin Fe^{59} and albumin I^{131} were added to the serum. In the schematic diagram of Fig. 1, it is seen that radioactivity is associated with protein that is essentially homogeneous electrophoretically. Reference to the elution profile (Fig. 2) for chromatogram 1 shows, however, that while the transferrin activity was chromatographically homogeneous, the I^{131} is to be found in three distinct peaks. Table I gives the electrophoretic classification of the proteins of fractions at the optical density peaks. In some fractions mobilities have been found to vary slightly from those for the classical electrophoretic components

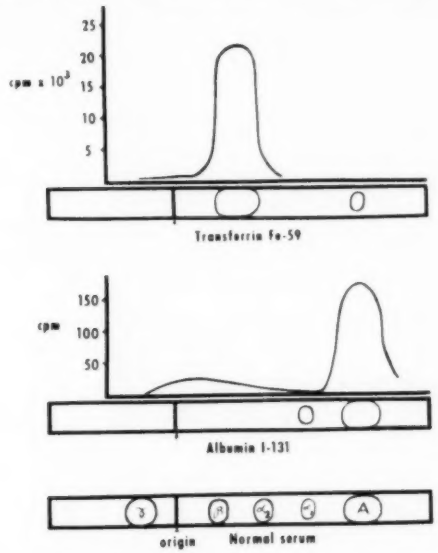

Fig. 1.—Schematic comparison of electrophoretic mobility and radioactivity of transferrin Fe^{59} and albumin I^{131} with normal serum.

of intact serum, but in this table and succeeding ones, assignments have been made for the serum protein component which corresponds most closely. Of interest is the fact that the gamma globulins appeared in a single protein peak under these elution conditions while the beta globulins and albumins were subfractionated to a considerable degree. Serologic testing (Table I) was done on individual fractions with group A_1 and group A_2 erythrocytes suspended in both saline and albumin. Activity was found to be well fractionated by each of the testing procedures. Reactions with A_1 cells were distributed into no

less than 4 distinct regions of the chromatogram; with A_2 cells, into 2 in saline and 3 in albumin. Of rather special interest are fractions 14 to 22 that reacted with A_1 cells in albumin but in no other test.

In chromatogram 2 (Fig. 3) the buffer system was changed so as to decrease initial ionic strength and to maintain an elevated pH for a longer period after introducing serum onto the column. This has retarded breakthrough of the gamma globulins and has resulted in improvement in their fractionation as evidenced by the electrophoretic mobilities recorded in Table II. With the larger quantity of serum used, the alpha globulins were better detected in the combined fractions and were well fractionated as were the beta globulins and albumins. Anti-A and anti-B activities were found in the same regions of the chromatogram. The Rh antibody was found distributed throughout the combined fractions, indicating great heterogeneity.

TABLE I. ELECTROPHORETIC AND SEROLOGIC PROPERTIES OF INDIVIDUAL FRACTIONS OF CHROMATOGRAM 1

FRACTION	ELECTROPHORETIC CLASSIFICATION	SEROLOGY			
		SALINE		ALBUMIN	
		A_1	A_2	A_1	A_2
Protein Charge		+	+	+	+
1-7		–	–	–	–
8, 9		+	+	–	–
10	β- + γ-globulin	+	+	+	+
11		+	–	+	+
12		–	–	+	+
13		+	–	+	+
14-22		–	–	+	–
23-39		–	–	–	–
40	Albumin + β-globulin	–	–	–	–
41-49		–	–	–	–
50	Albumin + β-globulin	–	–	–	–
51-54		–	–	–	–
55	Albumin + β-globulin	–	–	–	–
56-63		–	–	–	–
64		+	–	–	–
65	Albumin + β-globulin	–	–	–	–
66		–	–	–	–
67	Albumin	–	–	–	–
68-82		–	–	–	–
83	Albumin + α_2-globulin	–	–	–	–
84-99		–	–	–	–
100		–	–	–	–
101-104		–	–	–	–
105, 106		–	–	–	–
107-109		–	–	–	–
110-117		+	–	+	–
118-123		–	–	–	+
124-140		+	–	+	–
141-144		–	–	–	–
145-164		–	–	–	–

With a view toward finding conditions that would enhance adsorption of gamma globulins and thereby increase the opportunity for fractionation, a series of batch experiments were performed with various anions at pH 9.0. Glycine and borate were the most effective in increasing protein adsorption. The usefulness of borate is limited, however, by its rather low solubility. In developing chromatogram 3, a constant amount of glycine was maintained in all buffers while applying the usual gradients of increasing ionic strength and decreasing

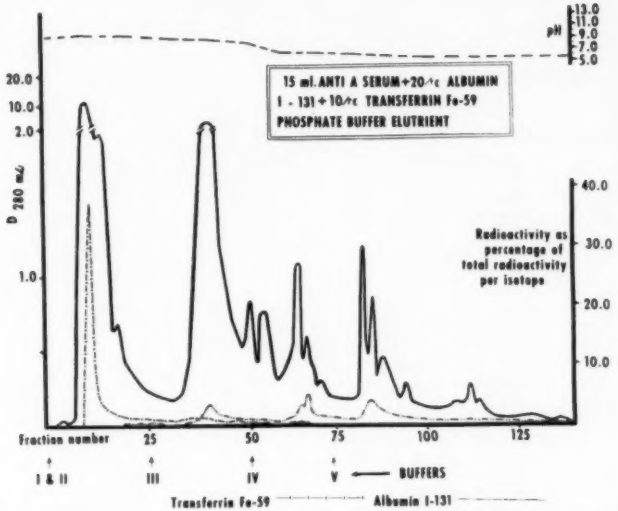

Fig. 2.—Chromatogram 1. Buffer system: constant NaCl, 0.025 M. In the following sequence the molarity of sodium phosphate and pH, respectively, are given: I, 0.005, 9.0; II, 0.010, 8.0; III, 0.020, 7.0; IV, 0.040, 6.0; V, 0.080, 5.0. Flow rate: 15 ml. per hour. Effluent volume: 1.8 L. Added proteins: 10 μc transferrin-Fe^{59} and 20 μc albumin I^{131} (both products of Abbott laboratories).

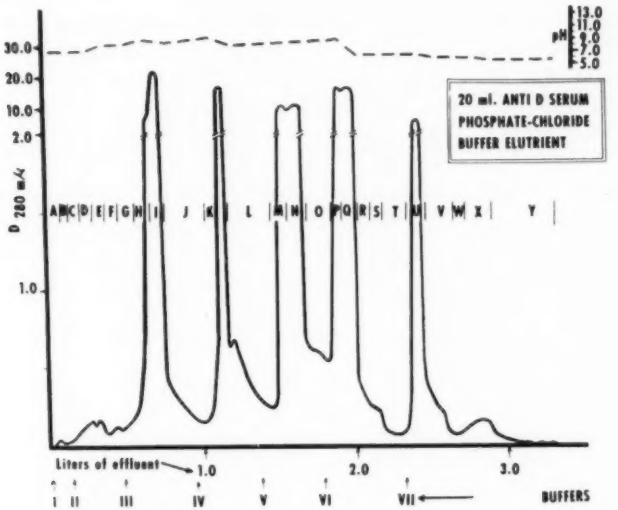

Fig. 3.—Chromatogram 2. Buffer system: constant NaCl, 0.0125 M. In the following sequence the molarity of sodium phosphate and pH, respectively, are given: I, no phosphate, 9.0; II, 0.001, 9.0; III, 0.010, 9.0; IV, 0.020, 8.0; V, 0.040, 7.0; VI, 0.080, 6.0; VII, 0.160, 5.0. Flow rate: 20 ml. per hour.

TABLE II. ELECTROPHORETIC AND SEROLOGIC PROPERTIES OF COMBINED FRACTIONS OF CHROMATOGRAM 2

COMBINED FRACTION	ELECTROPHORETIC CLASSIFICATION	SEROLOGY*					
		SALINE			INDIRECT COOMBS		
		A_1 Rh^-	B Rh^-	O Rh^+	A_1 Rh^-	B Rh^-	O Rh^+
Protein Charge		+	+	−	+	+	+
A	Undectectable	−	−	−	−	−	−
B	Undectectable	−	−	−	−	−	+
C	Undectectable	−	−	−	−	−	+
D	γ-globulin	−	−	−	−	−	+
E	Undetectable	−	−	−	+	−	+
F	Undetectable	−	−	−	+	−	+
G	Undetectable	−	−	−	+	−	+
H	γ-globulin	+	+	−	+	+	+
I	γ-globulin	−	+	−	+	+	+
J	γ-globulin	+	+	−	+	+	+
K	γ-globulin	−	−	−	+	+	+
L	β- + γ-globulin	−	−	−	+	−	+
M	Albumin + α_2- + β-globulin	+	+	−	+	+	+
N	Albumin	−	−	−	−	−	+
O	Albumin	−	−	−	+	−	+
P	Albumin + α_2-globulin	−	−	−	+	−	+
Q	Albumin + α_2-globulin	−	−	−	−	−	+
R	Albumin	−	−	−	−	−	+
S	Undetectable	−	−	−	−	−	+
T	Undetectable	−	−	−	−	−	+
U	Albumin + α_2-globulin	−	−	−	−	−	+
V-Y	Undetectable	−	−	−	−	−	−

*All control agglutination tests with type O, Rh^- cells were negative.

pH. The elution profile (Fig. 4) for serum Jo under these conditions shows better resolved protein peaks. The electrophoretic classification and serology on the combined fractions are given in Table III. As 0.015 M glycine has a lytic action on red cells, it is important that it be removed by dialysis or decreased by dilution before performing serologic tests.

TABLE III. ELECTROPHORETIC AND SEROLOGIC PROPERTIES OF COMBINED FRACTIONS OF CHROMATOGRAM 3

COMBINED FRACTION	ELECTROPHORETIC CLASSIFICATION	SEROLOGY*					
		SALINE			INDIRECT COOMBS		
		A_1 Rh^-	B Rh^-	O Rh^+	A_1 Rh^-	B Rh^-	O Rh^+
Protein Charge		+	+	−	+	+	+
A-C	Undetectable	−	−	−	−	−	−
D	γ-globulin	+	+	−	+	+	+
E	Albumin + β- + γ-globulin	−	−	−	+	+	+
F	β-globulin	−	−	−	+	+	+
G	Albumin	−	−	−	+	+	+
H	Albumin	−	−	−	−	−	+
I	Albumin	−	−	−	−	−	+
J	Albumin + α-globulin	−	−	−	−	−	+
K	Albumin + α_2-globulin	+	+	−	+	+	+
L	Undetectable	−	−	−	−	−	−
M	Undetectable	+	+	−	+	+	−
N-R	Undetectable	−	−	−	−	−	−

*Control agglutination tests with group O, Rh^- cells were all negative.

From calculations based on optical densities of individual fractions protein recoveries have consistently been of the order of 90 per cent.

DISCUSSION

In the work reported here, there has been ample verification of the claims of Peterson and Sober and their associates as to the efficacy of DEAE-cellulose in resolving complex mixtures of proteins. Protein peaks have been sharpened by using a modified buffer system, the principal features of which include a wider range of pH (9.0 to 4.5), the inclusion of glycine, and more gradual gradients of both pH and ionic strength in the earlier part of the chromatogram particularly. With the pH and ionic strength utilized in the first part of the elution cycle for chromatograms 2 and 3, it has been possible to prevent breakthrough of the gamma globulins and consequently to effect their subfractiona-

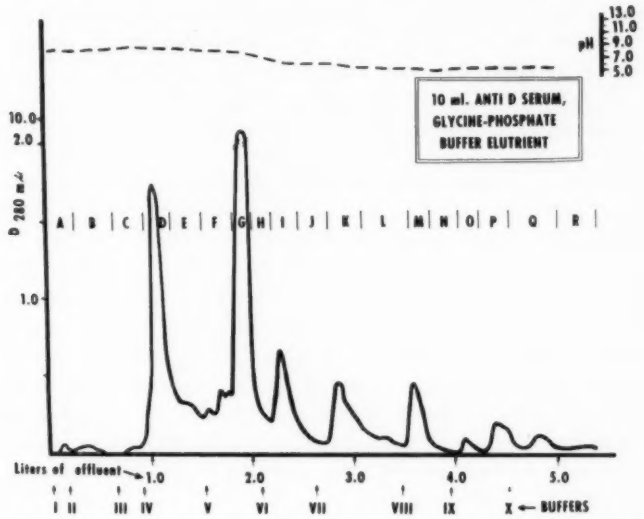

Fig. 4.—Chromatogram 3. Buffer system: constant glycine, 0.015 M. In the following sequence the molarity of sodium phosphate and pH, respectively, are given: I, no phosphate, 9.0; II, 0.001, 9.0; III, 0.010, 9.0; IV, 0.020, 8.0; V, 0.040, 7.0; VI, 0.080, 6.0; VII, 0.160, 5.0; VIII, 0.320, 5.0; IX, 0.500, 5.0; X, same as IX with NaCl increased to 0.100 M. Flow rate: 20 ml. per hour.

tion. It is of interest that albumin is distributed among several chromatographic peaks and that albumin-I^{131} was found by assay of radioactivity in three distinct peaks. Since completion of this work, Fahey and Steinfeld[8] studied radioactively labeled albumin chromatographically on DEAE-cellulose and have concluded that iodinated albumin differs significantly from native serum albumin. Sharp and associates[9] were perhaps the first to demonstrate electrophoretic heterogeneity for albumin, and since that time chromatographic heterogeneity has been demonstrated first by ion exchange[2, 10] and later by Tiselius and co-workers[11] with calcium phosphate gel. In fact each of the classical electrophoretic components of serum has been subfractionated by the chromatographic process herein described. Some of the protein peaks are homogeneous electrophoretically, while others are not. This finding points

to the importance of applying multiple techniques to protein fractionation, each making use of a different property of the protein molecule in order to achieve the ultimate in protein purity.

Serologic testing in this study has been concerned with antibodies to the ABO and Rh factors of human red blood cells. Considerable heterogeneity has been observed for each antibody studied, irrespective of specificity. Antibodies have been found in fractions for which electrophoretic mobilities ranging from that of albumin to that of gamma globulin were observed. Contrary to the widely held idea that antibodies are gamma globulins is the finding of serologic activity in fractions for which the only demonstrable electrophoretic component is albumin (e.g., fractions G, H, I, Table III, and fractions N, O, and R, Table II). However, because of the great sensitivity of serologic reactions, one cannot rule out the possibility that fractions with antibody activity might contain gamma globulin in quantities too low to be detected by paper electrophoresis.

From work with the Rh system it has become evident that there are sera which, despite inability to agglutinate saline suspensions of erythrocytes, contain antibodies that may be demonstrated by other techniques. Hill and Haberman[12, 13] have pointed out that these nonagglutinating antibodies are of two different types. In their nomenclature the agglutinoid is the blocking antibody as originally characterized by Wiener[14] and Race[15] and may be demonstrated by its ability to block agglutination in saline, by prozone phenomena, and by the Coombs reaction. The cryptagglutinoid is also detected by the Coombs test, by agglutinating red cells in various colloidal media, or by agglutinating enzyme-treated red cells, but it does not block agglutination. Of special interest here is the separation of anti-A of the cryptagglutinoid type from anti-A agglutinin in fractions 14 to 22 of chromatogram 1 and again in the combined fractions of chromatograms 2 and 3. In the latter cases, the nonagglutinating anti-A and anti-B may not unequivocally be classified as cryptagglutinoid, but it is presumed to be so because of lack of evidence of the presence of agglutinoid in the original serum. Of further interest from the standpoint of showing the high resolving power of this chromatographic technique are the fractions of chromatogram 1 which reacted positively with A_1 cells but not A_2, indicating a separation of anti-A from the more highly specific anti-A_1. From the data which have been presented, one is drawn toward the conclusion that antibodies of a given specificity are heterogeneous both serologically and physicochemically.

SUMMARY

Several different buffer systems have been examined for their efficacy in producing well-resolved protein patterns in chromatograms of human serum on DEAE-cellulose. A glycine-phosphate system with gradients of increasing ionic strength and decreasing pH has been shown to be particularly effective. Each of the classical electrophoretic components of serum has been subfractionated. Antibodies reacting with human erythrocytes (anti-A, anti-B, anti-D) have also been fractionated, and evidence has been presented for resolution of

anti-A from anti-A_1 and the separation of cryptagglutinoid from agglutinin. The use of radioactively labeled proteins has further aided evaluation of resolution.

REFERENCES

1. Peterson, E. A., and Sober, H. A.: Chromatography of Proteins. I. Cellulose Ion-exchange Adsorbents, J. Am. Chem. Soc. **78**: 751, 1956.
2. Sober, H. A., Gutter, F. J., Wyckoff, M. M., and Peterson, E. A.: Chromatography of Proteins. II. Fractionation of Serum Protein on Anion-exchange Cellulose, J. Am. Chem. Soc. **78**: 756, 1956.
3. Mitz, M. A., and Yanari, S. S.: Chromatography of Proteins on Cellulose Anion-Exchangers Using Water-Carbon Dioxide Systems, J. Am. Chem. Soc. **78**: 2649, 1956.
4. Ellis, S., and Simpson, M. E.: The Chromatography of Growth Hormone on Cellulose Derivatives, J. Biol. Chem. **220**: 939, 1956.
5. Lewis, U. J., and Thiele, E. H.: Isolation of a Pancreatic Insulinase, J. Am. Chem. Soc. **79**: 755, 1957.
6. Humphrey, J. H., and Porter, R. R.: Reagin Content of Chromatographic Fractions of Human Gamma-Globulin, Lancet **272**: 197, 1957.
7. Fahey, J. L., McCoy, P. F., and Goulian, M.: Chromatography of Serum Proteins in Normal and Pathologic Sera: The Distribution of Protein-Bound Carbohydrate and Cholesterol, Siderophilin, Thyroxin-Binding Protein, B_{12}-Binding Protein, Alkaline and Acid Phosphatases, Radio-Iodinated Albumin and Myeloma Proteins, J. Clin. Invest. **37**: 272, 1958.
8. Fahey, J. L., and Steinfeld, J. L.: Chromatographic Differences Between Radioiodinated Albumin Preparations and Normal Human Serum Albumin, Proc. Soc. Exper. Biol. & Med. **97**: 281, 1958.
9. Sharp, D. G., Cooper, G. R., Erickson, J. O., and Neurath, H.: The Electrophoretic Properties of Serum Proteins; II. Observations on Fractions of Crystalline Horse Serum Albumin, J. Biol. Chem. **144**: 139, 1942.
10. Boman, H. G., and Westlund, L.: Protein Chromatography on an Anion-Exchange Resin, Arch. Biochem. & Biophys. **64**: 217, 1956.
11. Tiselius, A., Hjerten, S., and Levin, O.: Protein Chromatography on Calcium Phosphate Columns, Arch. Biochem. & Biophys. **65**: 132, 1956.
12. Hill, J. M., and Haberman, S.: The Rh Factor; Analysis of a Routine Rh Testing Service, Texas State J. Med. **42**: 193, 1946.
13. Hill, J. M., Haberman, S., and Jones, F.: Hemolytic Rh Immune Globulins: Evidence for a Possible Third Order of Antibodies Incapable of Agglutination or Blocking, Blood **3**: Spec. Issue No. 2, pp. 80-100, 1948.
14. Wiener, A. S.: A New Test (Blocking Test) for Rh Sensitization, Proc. Soc. Exper. Biol. & Med. **56**: 173, 1944.
15. Race, R. R.: An Incomplete Antibody in Human Serum, Nature **153**: 771, 1944.

PROTEIN FRACTIONATION

II. Chromatography of Serum Proteins of Patients With Acute Leukemia and Multiple Myeloma

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USING the glycine-phosphate eluting system described in the preceding paper[1] for developing serum protein chromatograms on DEAE-cellulose, attention is here turned to examination of pathologic sera. A technique is featured for comparing the patient's serum with a normal serum by simultaneously developing two chromatograms from the same buffer reservoir. Modifications of the glycine-phosphate buffers and their effect on chromatographic resolution are described. In addition to characterizing chromatograms by optical density measurements, electrophoresis, and serology as were previously used, there have been included also studies with the diphenylamine reaction, lipid staining, determination of proconvertin, and ultracentrifugal data.

MATERIALS AND METHODS

Pertinent Patient History.—Patient Br was a 64-year-old white woman who was diagnosed on Jan. 9, 1957, as having acute granulocytic leukemia. Before institution of therapy, serum was collected for chromatography. Except where indicated serum samples were chromatographed within 24 hours after collection and were maintained under refrigeration without freezing. Patient Wi was a 4½-year-old white boy first seen on May 6, 1957. He was diagnosed at that time as having acute lymphocytic leukemia and was given transfusions of 250 ml. whole blood on May 6 and 7, 1957. The sample studied chromatographically was collected on May 8, 1957, before initiating antileukemia therapy. Patient Pe was a 3-year-old white boy with acute lymphocytic leukemia. He was in a special group of leukemia patients in whom bone marrow transplants were attempted following extensive irradiation with colloidal yttrium^{90} preparations. The clinical studies have been reported elsewhere.[2] Pe had been under treatment in the clinic of this institute for one year, during which time two remissions were obtained, one with Methotrexate treatment and the other following combined therapy with 6-mercaptopurine and prednisolone. The latter drug was continued throughout the period of irradiation. A plasma transfusion on March 6, 1958, was the last given prior to the period of study. The first serum sample studied was collected on March 11, 1958, before administration of $Y(OH)_3$-Y^{90} at a dose level of 3.5 mc. per kilogram body weight. On March 21, 1958, a second serum sample was collected before giving the bone marrow graft, permitting observation of changes in the serum proteins due to the hard beta ray (2.18 Mev.) of the yttrium, which had in this 10-day interval decayed through 4 half-lives. The first serum sample was stored frozen

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until after collection of the postirradiation sample; then the two were studied simultaneously. Included in this study are two cases of multiple myeloma. The first of these (Patient Ra) was a 65-year-old white woman who had been under treatment in the Wadley Clinic for 3 years. At the time of collection (March 21, 1957), the patient was receiving urethane. She had also received prednisolone, which was discontinued on March 12, 1957, and a transfusion of whole blood on March 2, 1957. Patient Ch was a 68-year-old man diagnosed as having multiple myeloma 1½ months prior to collection on June 3, 1957. At that time he was under treatment with urethane and prednisolone. Serum samples from normal individuals used for comparison with the pathologic sera were obtained from blood bank donors.

Preparation of the Chromatogram.—Preparation of the adsorbent and serum samples and development of the chromatogram were by techniques previously described.[1] An innovation introduced here will be referred to as the double column technique. To facilitate comparison of normal and pathologic sera, they have been chromatographed simultaneously on two columns prepared under identical conditions and eluted by buffer flowing from a single mixing chamber. The latter has been accomplished most easily by attaching a Y tube to the outlet at the bottom of a 1 L. Wolff bottle and then connecting the other two branches of the Y to the two columns. An alternative technique is to use an Erlenmeyer flask as the mixing chamber, an inverted bottle for addition of each of the buffers, and two siphon tubes leading to the columns. The columns were 22 mm. in diameter and contained about 20 Gm. of adsorbent packed to a height of 25 to 30 cm. An exception was the experiment with serum Pe in which, because of the reduced sample size, the column had a 10 mm. diameter and adsorbent height of 15 cm.

Evaluation of the Chromatogram.—Optical density and electrophoretic migration were measured as previously indicated. Electrophoresis of serum proteins of Patient Pe was quantitated with the Spinco Analytrol densitometer. Saline tests for anti-A and anti-B were performed on combined fractions (obtained by pooling successive chromatographic fractions, subjecting them to dialysis versus distilled water, lyophilizing them, and redissolving the protein in physiologic saline). In one experiment, tests for anti-A and anti-B were conducted with papain-treated erythrocytes (R.B.C.), using the modified procedure of Hill, Kurtar, and Soules.[3] Two volumes of 0.1 per cent papain activated with cysteine and disodium ethylenediaminetetraacetate (EDTA) were added to 1 volume of washed, packed red blood cells and incubated for 2 minutes at 37° C. The red blood cells were then washed in saline, and one drop of a 2 per cent suspension of these cells (in 0.9 per cent NaCl) was mixed with one drop of the test solution. After 2 minutes' incubation at 37° C., the tubes were centrifuged and examined microscopically. Location of lipids in the combined fractions has been by the Sudan black staining method of Swahn.[4] Proconvertin assays were by the method of Owren.[5] The Dische[6] diphenylamine reaction was performed on trichloroacetic acid filtrates of the combined fractions, and the visible absorption spectrum for the colors that developed was determined by scanning with the Beckman DK-2 spectrophotometer. Ultracentrifugation was conducted in the Spinco Model E ultracentrifuge with analytical rotor A on serum samples diluted with 0.9 per cent NaCl to a protein concentration of approximately 1 per cent. Enlarged prints were prepared from Schlieren patterns, and the area under each peak was determined by manually tracing the curve with the Analytrol and making use of its automatic integration feature. From these areas, the percentage of serum protein associated with each sedimentation peak was calculated.

RESULTS

Pathologic sera were obtained from selected patients seen in the Wadley Clinic with acute leukemia and multiple myeloma. The sera have been chromatographed on DEAE-cellulose with modified glycine-phosphate buffers.

Data presented here have been selected both for illustration of certain generalities concerning the chromatograms and also for novel features to which attention may be drawn for a given pathologic serum.

The chromatogram for serum from acute granulocytic leukemic patient Br is seen in Fig. 1, and evaluation of fractionation by studies of the combined fractions is given in Table I. The distribution through the chromatogram of the various electrophoretic classes in the original serum is rather complex. The first several peaks are found to be electrophoretically homogeneous, but the later fractions are mixtures showing 2 and sometimes 3 electrophoretic components. As noted previously[1] the mobilities vary somewhat from those observed for the proteins of intact serum. This kind of observation has also

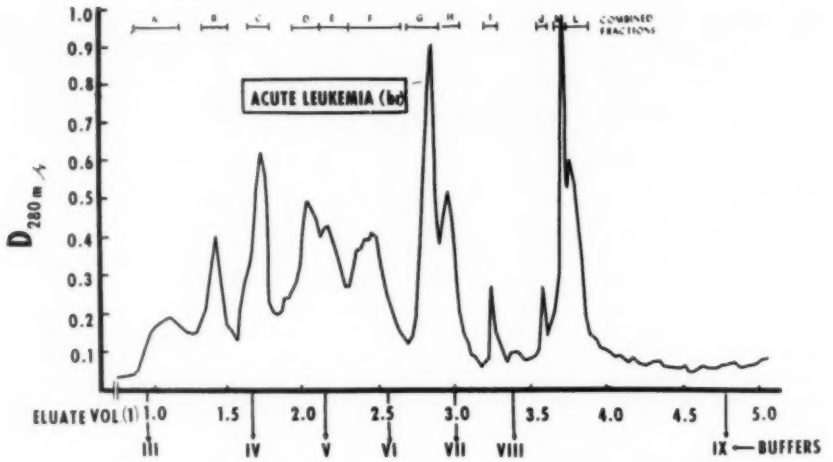

Fig. 1.—Chromatogram of 15 ml. serum Br (acute granulocytic leukemia). Buffer system: constant glycine, 0.015 M. In the following sequence the molarity of sodium phosphate and pH, respectively, are given: I, no phosphate, 9.0; II, 0.02, 8.0; III, 0.04, 7.0; IV, 0.08, 6.0; V, 0.16, 5.0; VI, 0.32, 5.0; VII, 0.50; 5.0 VIII, 1.00, 5.0; IX, same as VIII with 0.50 M NaCl added. Flow rate: 26 ml. per hour.

TABLE I. CHARACTERIZATION OF COMBINED FRACTIONS FROM CHROMATOGRAM OF SERUM BR (ACUTE GRANULOCYTIC LEUKEMIA)

COMBINED FRACTION	ELECTROPHORETIC CLASSIFICATION	SEROLOGY*			DIPHENYLAMINE REACTION†
		A_1 CELLS	A_2 CELLS	B CELLS	
A	γ-globulin	+	+	−	Green
B	β-globulin	+	−	−	Green
C	Albumin	+	−	−	Green
D	Albumin	−	−	−	Green
E	Albumin	−	−	−	Green
F	Albumin + α_1- + α_2-glob.	−	−	−	Violet
G	Albumin + α_2- + β-glob.	−	−	−	Violet
H	α_2- + γ-globulin	+	+	+	Violet
I	α_2- + β-globulin	+	+	+	Violet
J-L	Undetected	−	−	−	Green

*Tests done in saline; control tests with group O red cells were negative.
†Absorption maxima: green, 469 mμ; violet, 530 mμ.

been made by Sober[7] and Fahey.[8] Serologic studies with saline suspended red blood cells show isoagglutinins appearing in two distinct and well-separated portions of the chromatogram. The diphenylamine test gave two colors, yellow-green and violet with absorption maxima at 469 and 530 mμ, respectively. The green color appeared in fractions A to E and again in fractions J to L while the violet color appeared in successive combined fractions (F to I) representing 4 different chromatographic peaks.

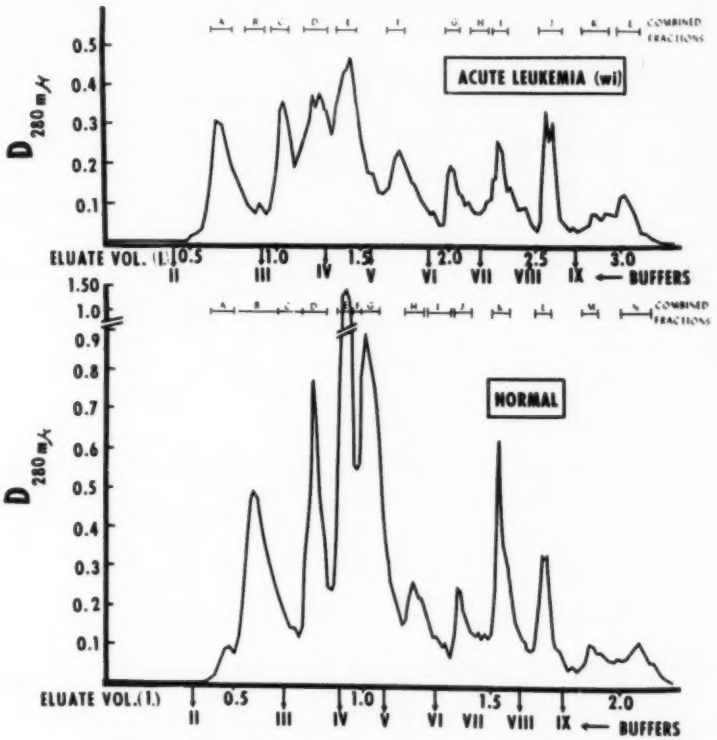

Fig. 2.—Double column chromatogram of 10 ml. serum samples from normal G105 and Patient Wi (acute lymphocytic leukemia) sera. Buffer system: constant glycine, 0.015 M. In the following sequence the molarity of sodium phosphate and pH, respectively, are given: I, no phosphate, 9.0; II, 0.02, 8.0; III, 0.04, 7.0; IV, 0.06, 6.5; V, 0.08, 6.0; VI, 0.12, 5.5; VII, 0.16, 5.0; VIII, 0.32, 5.0; IX, 0.50, 5.0. Flow rate: normal serum, 12 ml. per hour; serum Wi, 18 ml. per hour.

Chromatograms for other pathologic sera to be presented here were obtained by the double column technique. The chromatogram for one of these (serum Wi, acute lymphocytic leukemia) is seen in Fig. 2. Because of differences in flow rate for the two columns, different scales have been chosen to represent the effluent volume along the horizontal axis; the scales have been selected, however, so that protein peaks emerging from the column following

TABLE II. CHARACTERIZATION OF COMBINED FRACTIONS FROM DOUBLE COLUMN CHROMATOGRAM OF SERA FROM A NORMAL INDIVIDUAL AND PATIENT WI (ACUTE LYMPHOCYTIC LEUKEMIA)*

NORMAL							ACUTE LYMPHOCYTIC LEUKEMIA						
LIPID†	PROCONVERTIN (SEC.)	SEROLOGY‡ A_1	SEROLOGY‡ A_2	SEROLOGY‡ B	ELECTROPHORETIC CLASSIFICATION	COMBINED FRACTION	COMBINED FRACTION	ELECTROPHORETIC CLASSIFICATION	SEROLOGY‡ A_1	SEROLOGY‡ A_2	SEROLOGY‡ B	PROCONVERTIN (SEC.)	LIPID†
–	>100	–	–	–	Undetected	A							
–	>100	+	+	–	γ-globulin	B	A	γ-globulin	–	–	–	>100	–
–	>100	–	–	–	γ-globulin	C	B	γ-globulin	–	–	–	>100	–
–	93	–	–	–	Albumin + β-glob.	D	C	β_2- + γ-globulin	–	–	–	>100	–
++	96	–	–	–	Albumin + α_2- + β-glob.	E	D	Albumin + β-glob.	–	–	–	>100	+
+	82	–	–	–	Albumin	F							
++	>100	–	–	–	Albumin	G	E	Albumin	–	–	–	>100	++
+	67	–	–	–	Albumin + α_1-glob.	H	F	Albumin + α_1- + α_2-glob.	–	–	–	99	++
+	>100	–	–	–	Albumin + α_2-glob.	I							
+	87	–	–	–	Albumin + α_2-glob.	J	G	Albumin + α_2-glob.	–	–	–	93	+
							H	Albumin + α_2-glob.	–	–	–	>100	+
+	96	–	–	–	Albumin + α_2-glob.	K	I	Albumin + β-glob.	–	–	–	98	+
+	59	+	+	+	α_2- + β-globulin	L	J	α_1- + α_2-globulin	–	–	+	86	+
–	23	–	–	–	Undetected	M	K	Undetected	–	–	+	34	–
–	43	–	–	–	Undetected	N	L	Undetected	–	–	–	57	–

*Table II is arranged so that corresponding combined fractions are found along a single horizontal row.
†Key: ++, intense stain; +, moderate staining intensity; –, lipid absent.
‡Tests done in saline; control tests with group O red cells were negative.

addition of a given buffer will approximately line up with each other as they are viewed on the graphs. This same method of plotting has been used with each of the succeeding double column chromatograms. Despite the difference in flow rates, one finds close correspondence of the various protein peaks with respect to point of appearance in the chromatogram after addition of a given buffer; of possible interest is the reversal of relative peak heights for D and E of serum Wi as compared to the corresponding peaks E and G for the normal serum. Studies performed on the combined fractions from these two columns are summarized in Table II. The table is arranged so that data for corre-

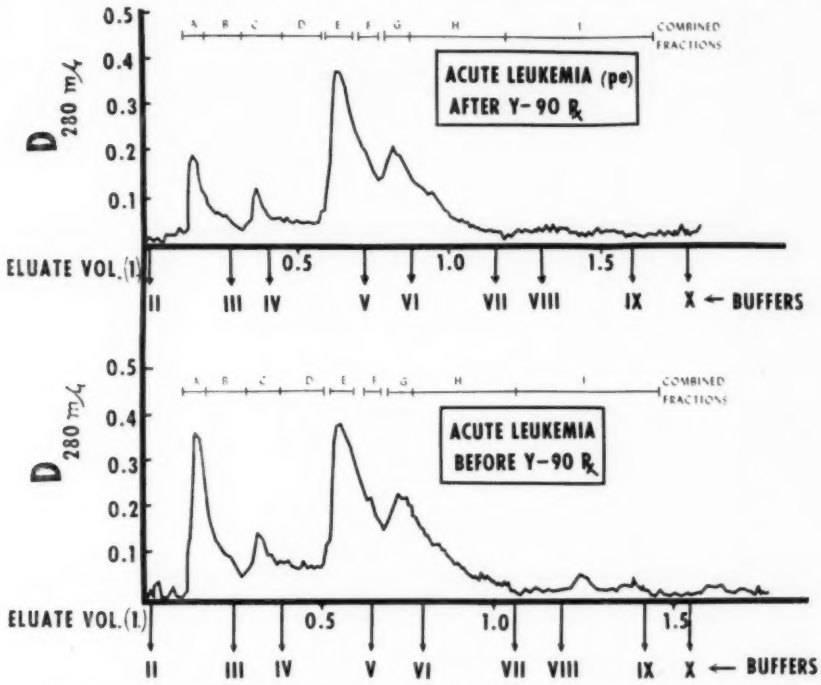

Fig. 3.—Double column chromatogram of 3 ml. serum samples from Patient Pe (acute lymphocytic leukemia) before and after treatment with $Y(OH)_3$-Y^{90}. Buffer system: constant glycine, 0.015 M; in the following sequence the molarity of sodium phosphate and pH, respectively, are given: I, no phosphate, 9.0; II, 0.02, 8.5; III, 0.04, 8.0; IV, 0.06, 7.5; V, 0.08, 7.0; VI, 0.10, 6.5; VII, 0.20, 6.0; VIII, 0.30, 5.5; IX, 0.50, 5.0; X, 1.00, 4.5. Flow rate: before $Y(OH)_3$-Y^{90}, 16 ml. per hour; after $Y(OH)_3$-Y^{90}, 19 ml. per hour.

sponding combined fractions for the two sera are found along a single horizontal row. Examination of the electrophoretic data shows that the distribution of the electrophoretic components in the two chromatograms closely parallel each other. Some differences for corresponding fractions are noted, however; normal fraction D contains albumin with β-globulin while Wi fraction C has γ-globulin accompanying the β-globulin. Another difference is seen in the

appearance of α_2-globulin in normal fraction E and its absence in Wi fraction D. It is noted that the homogeneous β-globulin fraction of Table I (fraction B) is not observed in this double column experiment. This difference reflects at least in part the number of individual fractions across one of the chromatographic peaks that were pooled to obtain the combined fraction for electrophoretic characterization. In the serologic studies anti-B activity was the

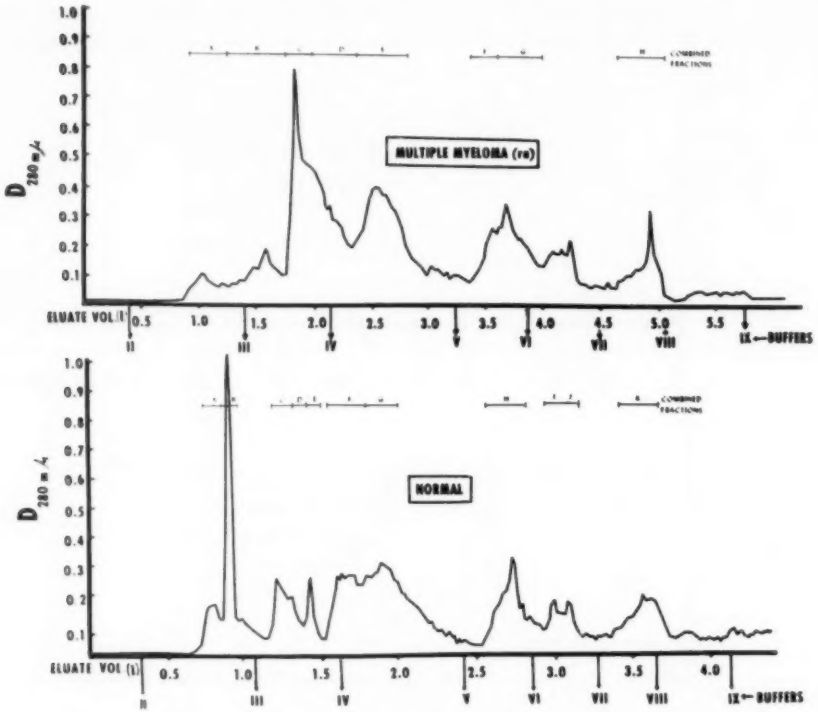

Fig. 4.—Double column chromatogram of 10 ml. serum samples from normal G103 and Patient Ra (multiple myeloma) sera. Buffer system: constant glycine, 0.015 M. In the following sequence the molarity of sodium phosphate and pH, respectively, are given: I, no phosphate, 9.0; II, 0.02, 8.0; III, 0.04, 7.0; IV, 0.08, 6.0; V, 0.16, 5.0; VI, 0.32, 5.0; VII, 0.50, 5.0; VIII, 1.00, 5.0; IX, same as VIII with 1.00 M NaCl added. Flow rate: Normal serum, 18 ml. per hour; serum Ra, 25 ml. per hour.

same for both columns, but anti-A was not detected in the fractions from serum Wi. The anti-A activity for the normal serum, however, had essentially the same distribution as that found in the chromatogram of serum Br. Proconvertin activity was found in corresponding combined fractions, M for the normal serum and K for serum Wi. Lipid distribution as determined by Sudan black staining was also the same for both sera, appearing through the mid-portion of the chromatogram and being absent in the early and late fractions.

Studies on serum samples from patient Pe before and 10 days after administration of $Y(OH)_3$-Y^{90} are recorded in Table III. Electrophoretically the serum proteins show a shift in distribution with decreases in the albumin and γ-globulin and concomitant increases in the α_2- and β-globulins. From ultracentrifugal data it is seen that sedimentation constants for the 3 groups of

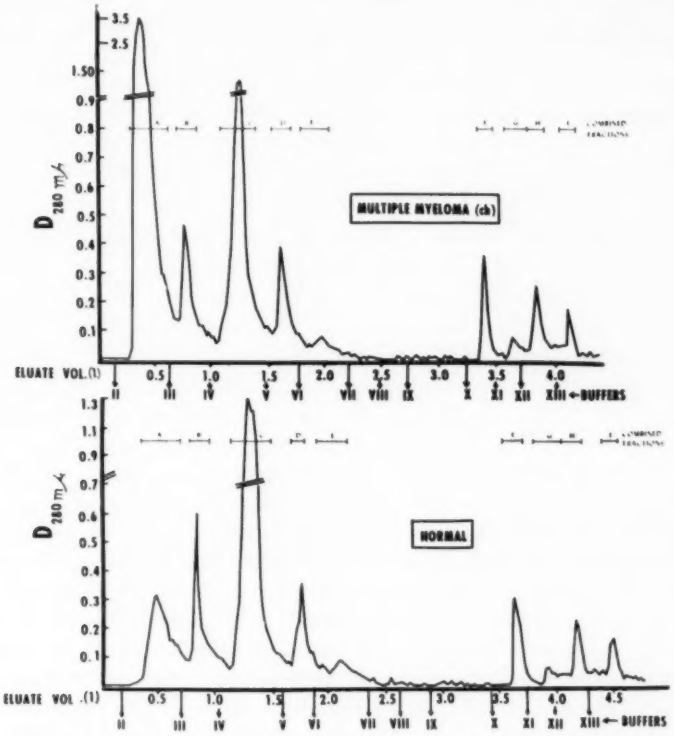

Fig. 5.—Double column chromatogram of 10 ml. serum samples from normal G107 and Patient Ch (multiple myeloma) sera. Buffer system: constant glycine, 0.015 M. In the following sequence the molarity of sodium phosphate and pH, respectively, are given: I, no phosphate, 9.0; II, 0.02, 8.5; III, 0.04, 8.0; IV, 0.06, 7.5; V, 0.08, 7.0; VI, 0.10, 6.5; VII, 0.12, 6.0; VIII, 0.14, 5.5; IX, 0.16, 5.5; X, 0.32, 5.0; XI, 0.50, 5.0; XII, 1.00, 4.5; XIII, same as XII with 0.50 M NaCl added. Flow rate: normal serum, 21 ml. per hour; serum Ch, 20 ml. per hour.

proteins have remained unchanged but that there has been some shift of proteins from the 7S to the 4S group. (The sedimentation constants as reported are uncorrected for viscosity.) Anti-A and anti-B titers were obtained both with saline suspended R.B.C. and papain-treated R.B.C.; by both techniques titers were decreased in the postirradiation sample. Chromatographically the 2 sera show an almost exact correspondence except for the quantity of protein in the first large peak (Fig. 3). Corresponding combined fractions gave identical electrophoretic patterns. Diphenylamine reactions gave essentially

TABLE III. STUDIES ON PE SERA BEFORE AND TEN DAYS AFTER ADMINISTRATION OF COLLOIDAL $Y(OH)_3$-Y^{90}

STUDY	BEFORE $Y(OH)_3$-Y^{90}		AFTER $Y(OH)_3$-Y^{90}	
Electrophoresis	Per Cent Protein		Per Cent Protein	
Albumin	53.6		45.3	
Alpha$_1$ globulin	5.3		5.3	
Alpha$_2$ globulin	12.1		17.9	
Beta globulin	9.2		16.8	
Gamma globulin	19.8		14.7	
Ultracentrifugation	S_{20}*	Per Cent Protein	S_{20}*	Per Cent Protein
	15.0	4.4	15.0	4.6
	5.5	23.5	5.8	16.1
	3.6	72.1	3.7	79.3
Serologic titers	Saline	Papain	Saline	Papain
Anti-A	512	> 2,000	128	1,000
Anti-B	128	1,000	32	256

*Uncorrected for viscosity.

the same distribution of colored products as was observed with serum Br (Table I); fractions E to H from both Pe sera gave the violet color with an absorption maximum at 530 mμ, and the other fractions gave the yellow-green color. For both sera, anti-A and anti-B were detected only in that region of the chromatogram corresponding to combined fraction I when the individual fractions were examined by the saline test and with papain-treated R.B.C. The small samples (3 ml.) chromatographed in this experiment may account for the failure to find antibodies elsewhere in the chromatogram.

The two multiple myeloma patients were chosen because of an interesting difference in electrophoretic pattern. Serum Ch had a large increase in γ-globulin; while serum Ra had hypogammaglobulinemia and myeloma protein of a mobility intermediate between that of γ- and β-globulins. Chromatographic comparison of these sera with normal sera by the double column technique is to be found in Figs. 4 and 5. Fractions A and B for the normal serum of Fig. 4 represent an appreciable quantity of protein (γ-globulin), but the corresponding peaks for serum Ra are considerably smaller. On the other hand, fraction C of serum Ra is much larger than the corresponding peak in the chromatogram of the normal serum. The protein of fraction C (serum Ra) was found to have the same electrophoretic mobility as the abnormal protein of the original serum, i.e., mobility between that of γ- and β-globulins. The lipid distribution for both sera was the same as that recorded in Table II; electrophoretic components were distributed in essentially the same way as indicated in previous tabulations. Isoagglutinins were found in fractions A, B, C, I, and J from the normal serum, but, because of the low titer in serum Ra, they were not detected in the combined fractions obtained therefrom. Proconvertin assays failed to reveal this enzyme. The abnormally large peak (fraction A) in the chromatogram of serum Ch (Fig. 5) contains protein with typical γ-globulin mobility, as does the corresponding peak in the normal serum chromatogram. With the gradual gradients of pH and ionic strength utilized in this experiment, the sharpest and most symmetrical protein peaks

yet seen in our studies were obtained. Electrophoretic heterogeneity in the combined fractions was the same as in other cases, however. For both sera, isoagglutinins were found in fraction F only.

DISCUSSION

In the course of these studies changes in the gradients of pH and ionic strength have been used to determine their effect on protein resolution. The general tendency was to decrease the steepness of the gradients, and this has been accomplished by using an increased number of buffers differing from the preceding buffer by smaller and smaller increments. This adjustment of eluting conditions has led to increased sharpness and symmetry of the chromatographic peaks as evidenced by the chromatograms of Fig. 5. In that experiment, which has now been adequately duplicated, the largest number of buffers was used with the smallest jumps in pH and ionic strength. It is apparent that buffers VII, VIII, and IX, which eluted no protein from the column, may be eliminated without affecting resolution.

Distribution of the serum electrophoretic components through the chromatogram is rather complex, but some generalizations may be made. The first protein peak which emerges from the column contains the bulk of the serum γ-globulin, and in this group of fractions it is free from other serum electrophoretic components. The beta globulins next begin to appear in the eluate, and, if one examines fractions in the immediate vicinity of the second protein peak, this protein will generally be electrophoretically homogeneous. As the number of fractions in this peak that are combined for electrophoretic examination is increased, one is able to detect the trailing edge of the γ-globulins and the leading edge of the albumins that are the next proteins eluted. The albumins give a rather complex elution pattern appearing in three or four chromatographic peaks. The first one or two of these peaks have most frequently been found free of globulins, but the others contain alpha globulins. Following the albumin peaks, one finds fractions with varying ratios of the globulins. The variability in this region of the chromatogram may be due in part to the fact that relatively small quantities of protein are involved, and, with the limitations that exist in detecting minor electrophoretic components on paper, a particular protein component may be missed. It has not been possible to characterize electrophoretically the protein of the last chromatographic peak despite the fact that judging from optical density and biuret determinations there should be enough protein to be visualized with bromphenol blue staining. Failure to take the dye may mean that the bulk of material in these fractions is relatively low molecular weight polypeptides; this point is currently under investigation in our laboratories. These findings are for the most part in agreement with those of Fahey,[5] and with the findings of Sober,[7] who used a somewhat simpler phosphate buffer system.

Isoagglutinin detection in this study has been primarily by saline agglutination. It has been possible to demonstrate the presence of anti-A in two widely separated regions of the chromatogram. Anti-A activity first appears

in the early chromatographic peaks which contain large percentages of γ-globulin and then again in the fractions following addition of the buffer which contains 0.32 M sodium phosphate at pH 5.0. Anti-B has been detected in similar portions of the chromatogram but with somewhat less consistency in the early combined fractions. The appearance of isoagglutinin activity in the combined fractions immediately following the 0.32 M Na phosphate, pH 5.0 buffer has been one of the most constant findings throughout this study. Electrophoretically the protein of these late, active fractions has been a mixture of globulins with beta globulins being found most consistently, although gamma globulin was also demonstrable in the chromatogram of serum Br (Table I).

The inferences that may be drawn from these studies are that the saline agglutinins are heterogeneous, that they are separable chromatographically, and that the segregated activities are different from each other electrophoretically. Further support of the idea of general antibody heterogeneity comes from a preliminary report of chromatographic studies involving a completely different chromatographic system using calcium phosphate (hydroxylapatite) as an adsorbent.[9] One note of caution should be introduced with respect to interpreting chromatographic fractionation of a biologic activity as rigorous *proof* of the heterogeneous nature of that activity. "Double zoning" as distinct from "tailing" has been pointed out by Boman[10] as a real source of error in interpreting chromatograms of protein mixtures. Additional bases are being sought for establishing as real or artifactual the apparent physicochemical heterogeneity of the blood group antibodies indicated by the chromatographic studies.

Reaction of the combined fractions with diphenylamine has shown carbohydrate to distribute throughout the various portions of the chromatogram. This broad distribution of carbohydrate is in agreement with the findings of Fahey and associates[8] who used the thymol-sulfuric acid method for detecting glycoprotein. Of particular interest in our own study is the location of the substance producing the violet color (absorption maximum, 530 mμ) with diphenylamine. In applying this reaction to serum samples of some 2,200 patients with a variety of pathologic conditions, Waldo and Zipf[11] found that individuals with leukoproliferative disorders gave the most intense color. They further claimed that in leukemia patients the color-producing substance increased in the serum throughout the course of the disease, irrespective of remission. More recently studies by Hess, Hahn, and Ayala[12] have indicated that the substance producing the purple color may be sialic acid, a common substituent of glycoproteins. This material has now on several occasions been found in the portion of the chromatogram in which the alpha globulins are prominent. That it is found in several chromatographic peaks suggests that it is bound to more than one particular protein.

The lipid staining studies have shown that the lipids are distributed through the mid-portion of the chromatogram and are absent from both the early and late fractions. From cholesterol determinations, Fahey[8] has again come to a conclusion with which our data are in substantial agreement.

Assay for proconvertin showed that an active material was eluted near the end of the chromatogram (Fig. 2). We have not at this point ruled out the possibility that the active substance may be thrombin which was detected by Sober and associates[7] in a roughly comparable region of the chromatogram.

Because of the known protein abnormality accompanying multiple myeloma, the sera of patients with this disease represent an interesting group for testing the potential of chromatography on DEAE-cellulose as a means of differentiating normal and pathologic sera. In this regard we have successfully differentiated dissimilar myeloma proteins as seen in Figs. 4 and 5 (also see Results). These studies add weight to the concept that multiple myeloma, although clinically a single disease state, may be differentiated at the molecular level by the kind of protein abnormality that is produced. Aside from peaks containing the myeloma proteins, other protein peaks from the normal and pathologic sera of a given double column experiment correspond closely both from the standpoint of the position of the peak in the chromatogram and the amount of protein which it contains. This close correspondence suggests a high degree of reproducibility for a given set of conditions.

Although many authors have suggested that there are serum protein changes in leukemia which may be detected electrophoretically, there are none which compare in magnitude with those observed in multiple myeloma. The double column experiment with leukemic serum reported here (Fig. 2) shows rather close correspondence with the corresponding normal pattern. Other leukemic sera which we have studied also closely parallel the normal pattern. The one exceptional serum is that of patient Br; with the large protein peaks appearing at the end of the chromatogram it stands as completely atypical among sera thus far investigated. Although Br had received no form of therapy at the time the serum was collected, most of the leukemia patients studied were under treatment. Whether this case is anomalous or whether there are protein abnormalities which occur in the natural history of acute leukemia that may be detected by this chromatographic method is being currently investigated.

SUMMARY

Glycine-sodium phosphate buffers have been examined for their efficacy in producing well-resolved chromatograms of serum proteins from columns of DEAE-cellulose. Gradient elution systems with small increments of increasing ionic strength and decreasing pH have produced chromatograms with the sharpest and most symmetric protein peaks. Saline agglutinating anti-A and anti-B have been found in two characteristic and well-resolved portions of the chromatogram, providing additional evidence for the concept of isoagglutinin heterogeneity. Although some generalizations are made, rather complex distributions have been observed for the classical electrophoretic components of serum. Lipid-containing fractions are distributed through the mid-portion of the chromatogram. Carbohydrate-containing fractions are widely distributed; of interest is the identification of sialic acid in several protein peaks. Chromatographic characterization by a double column technique of sera of patients with acute leukemia and multiple myeloma is reported. Myeloma proteins (obtained from

two different patients) with dissimilar electrophoretic mobilities have been further differentiated chromatographically. Chromatograms obtained on leukemic sera have not differed greatly from those for normal sera except for an untreated case with a highly individual pattern. Serum protein changes arising from massive irradiation with $Y(OH)_3$-Y^{90} of an acute leukemic patient (in preparation for a bone marrow transplant) are also reported.

REFERENCES

1. Speer, R. J., Prager, M. D., Kelley, T. F., and Hill, J. M.: Protein Fractionation: I. Chromatography of Human Serum Proteins on DEAE-Cellulose With Special Reference to Blood Group Antibodies, J. Lab. & Clin. Med. **54**: 685-693, 1959.
2. Hill, J. M., Loeb, E., Smith, A. L., and Speer, R. J.: Treatment of Acute Leukemia, VIIth Congress of the International Society of Hematology, p. 113, 1958.
3. Hill, J. M., Kurtar, K., and Soules, D. E.: Personal communication.
4. Swahn, B.: Studies on Blood Lipids, Scandinav. J. Clin. & Lab. Invest. **5**: Suppl. 9, 1953.
5. Owren, P. A., and Aas, K.: The Control of Dicumarol Therapy and the Quantitative Determination of Prothrombin and Proconvertin, Scandinav. J. Clin. & Lab. Invest. **3**: 201, 1951.
6. Dische, Z.: Some New Characteristic Color Tests for Thymonucleic Acid and a Microchemical Method for Determining the Same in Animal Organs by Means of These Tests, Mikrochemie **8**: 4, 1930.
7. Sober, H. A., Gutter, F. J., Wyckoff, M. M., and Peterson, E. A.: Chromatography of Proteins. II. Fractionation of Serum Protein on Anion-exchange Cellulose, J. Am. Chem. Soc. **78**: 756, 1956.
8. Fahey, J. L., McCoy, P. F., and Goulian, M.: Chromatography of Serum Proteins in Normal and Pathologic Sera: The Distribution of Protein-Bound Carbohydrate and Cholesterol, Siderophilin, Thyroxin-Binding Protein, B_{12}-Binding Protein, Alkaline and Acid Phosphatases, Radio-Iodinated Albumin and Myeloma Proteins, J. Clin. Invest. **37**: 272, 1958.
9. Prager, M. D., Speer, R. J., and Bryan, S.: Chromatography of Human Blood Group Antibodies on Hydroxylapatite and DEAE-Cellulose, Abstr. 134th National Meeting, American Chemical Society, September, 1958, p. 58C.
10. Boman, H. G.: Ion Exchange Chromatography of Proteins and Some Applications to the Study of Different Phosphoesterases, Swedish Government Publication, April, 1958 (from Almquist and Wiksell, D. Sc. Thesis, University of Uppsala, 1958).
11. Waldo, A. L., and Zipf, R. E.: The Diphenylamine Test on Leukemic Sera, Experientia **10**: 48, 1954.
12. Hess, E. L., Hahn, J. W., and Ayala, W.: Structures Responsible for the Characteristic Purple Color Development in Diphenylamine Reaction With Serum, Proc. Soc. Exper. Biol. & Med. **91**: 528, 1956.

IMMUNOELECTROPHORESIS IN PATIENTS WITH HYPERPARATHYROIDISM

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IMMUNOELECTROPHORESIS is a relatively new method of analysis.[1] Its application in clinical practice is limited at present by the difficulty of the method and the necessity of using valuable animal antisera as detecting agents. The antisera react with the greatest possible number of antigens in human sera and produce clear precipitation lines. In some diseases such as mutiple myeloma,[2] acute polyarthritis,[3] and in macroglobulinemia of Waldenström,[4, 5] the method has yielded very valuable results.

We wished to investigate the immunoelectrophoretic pattern of patients with hyperparathyroidism in order to ascertain whether changes in the protein structure occur in this disease. Since immunoelectrophoresis is a very sensitive analytical method, we thought that it might reveal changes in protein structure which cannot be detected either by paper or moving boundary electrophoresis.

SUBJECTS AND METHODS

Immunoelectrophoric determinations were made on 5 patients with hyperparathyroidism. (4 women between 45 and 60 years of age and 1 man 43 years of age). All patients showed skeletal alterations typical of this disease and the diagnoses were also supported by biochemical examinations. All 5 patients were operated upon; 4 of them had adenomas of the parathyroid gland, and the fifth patient had hyperplasia of the parathyroid glands. In 3 of these patients, immunoelectrophoresis was carried out at intervals of 3 to 8 months following the operations.

Blood samples were taken from the patients while they were fasting and the serum was preserved with 100 units per milliliter of penicillin and streptomycin. Blood of healthy individuals of the same age group was collected simultaneously under the same conditions.

Antisera.—(1) Rabbit antiserum obtained by immunizing rabbits with serum from healthy human beings according to the method of Cleve,[3] (anti-N serum). (2) Rabbit antiserum obtained by immunization with mixed serum of 3 patients with hyperparathyroidism. (anti-R serum). The sera were preserved by means of Merthiolate (1: 10,000) and kept in the frozen state at -10° C.

Antigens.—Sera of 5 patients with hyperparathyroidism and sera of 5 healthy individuals.

Method.—Immunoelectrophoresis was carried out in 1.5 per cent Difco agar gel on glass plates (18 by 5 cm.) with a Veronal buffer at pH 8.6 (ionic strength 0.05), using the method of Williams and Grabar[1] and Wunderly.[2] In the first case the serum examined was mixed with agar at 45° C. and then pipetted into a starting hole; in the second case the serum was diffused from the filter paper which was pressed to the surface of the agar plate. Each serum was examined in 2 concentrations: (1) the serum mixed with agar in a 1: 1 ratio and (2) diluted 5 times. The rabbit antisera were used in a concentrated form. The results obtained from both methods were identical.

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RESULTS

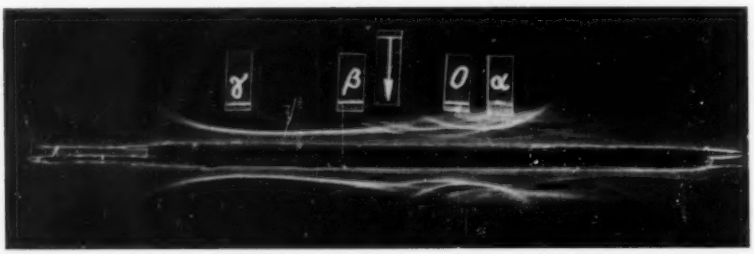
I. In all patients with hyperparathyroidism there is an additional line in the beta-1-globulin area which was absent in the control sera when anti-R sera are used (Figs. 1-4).

II. The anomalous line in the beta-1-globulin area is also visible on testing with 3 anti-N sera, whereas one anti-N serum does not reveal any difference between the spectrum of the pathologic and the control sera.

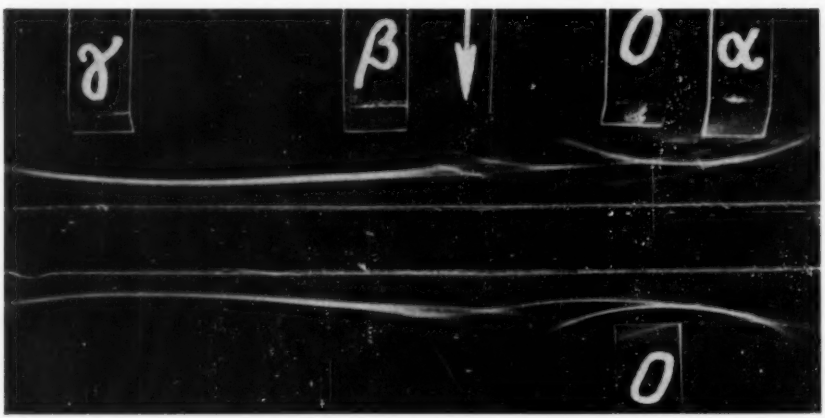
III. In each of the 3 patients suffering from adenoma of the parathyroid gland, the anomalous precipitation line was very marked; whereas in the patient with adenomatous hyerplasia of the parathyroid glands, it was barely visible.

IV. After the operation for removal of the adenomas, the anomalous line in the sera of the patients could not be detected by any of the antisera used.

V. The absorption experiments exclude the possibility of the presence of proteins with completely different antigenicity from that of normal serum proteins.



A.



B.

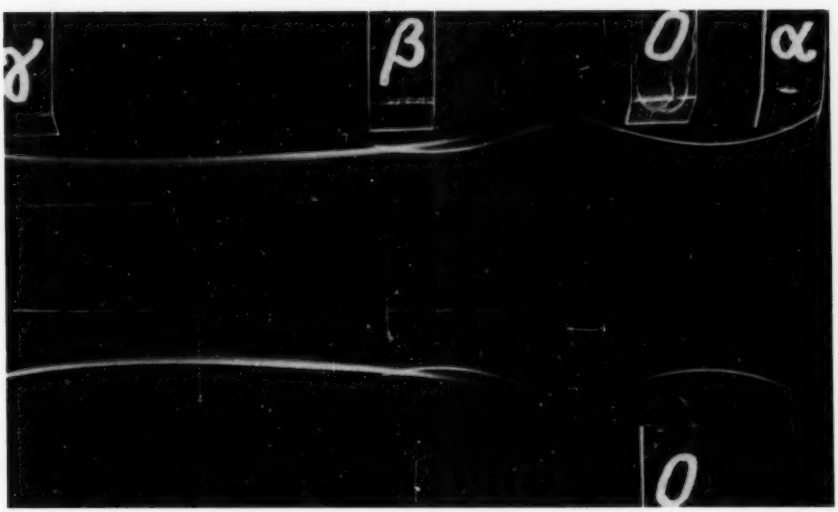
Fig. 1.—*A*, Immunoelectrophoresis of the serum of one patient before the operation compared with the normal serum. In the upper starting hole (*O*) is the serum of the patient with the adenoma of the parathyroid gland. In the lower starting hole is the serum of a blood donor. The rabbit anti-R serum is placed in the middle trough.

B, Detail of the identical immunoelectrophoretic plate.

DISCUSSION

It can be concluded from our experiments that the serum of patients with hyperparathyroidism contains a protein component which disappears after the removal of the parathyroid adenoma. The protein component has the same mobility as beta-1-globulin and the same, or nearly the same, antigenicity as some proteins present in normal serum under physiologic conditions. It is possible that one of the proteins of beta-1-globulin, present in normal serum in an amount insufficient to produce precipitation lines, multiplies, or that the beta-1-globulin is dissociated into 2 compounds of the same electrophoretic mobility but of different diffusion rates. In hyperparathyroidism not only the

A.



B.

Fig. 2.—*A*, Immunoelectrophoresis of the serum of the same patient after the operation compared with the normal serum. In the upper starting hole (*O*) is the serum of the patient after the operation for removal of the adenoma of the parathyroid gland. In the lower starting hole is the serum of a blood donor. The rabbit anti-R serum is placed in the middle trough. *B*, Detail of the identical immunoelectrophoretic plate.

mineral component of the skeleton is attacked but also the organic part which consists mostly of collagen and glyco-proteins. Bronsky and associates[6] stated that the total mucoprotein level in the serum of rats increased after the application of an injection of parathormone, but they did not detect any deviation in the serum mucoprotein level in hyperparathyroidism or hypoparathyroidism as compared with the control serum. Mucoproteins, however, appear in electrophoretic examinations in the area of the alpha-globulins, whereas we observed deviations in that of the beta-globulins.

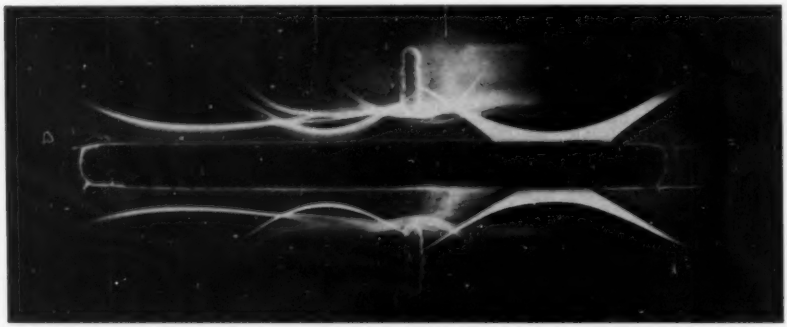


Fig. 3.—Immunoelectrophoresis of the serum of the patient with adenoma of the parathyroid gland on using antinormal rabbit serum. The upper starting hole contains the serum of the patient with adenoma of the parathyroid gland (serum mixed with agar 1:1). The lower starting hole contains the same serum mixed with agar 1:4. In the middle trough is rabbit anti-N serum.

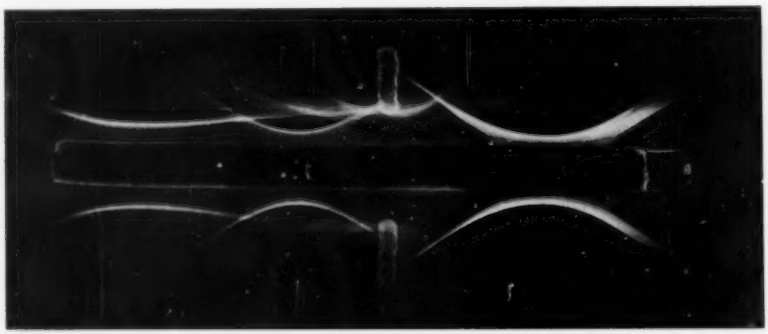


Fig. 4.—Immunoelectrophoresis of the serum of a healthy individual on using antinormal rabbit serum. The upper starting hole contains the serum of the healthy individual (serum mixed with agar 1:1). The lower starting hole contains the same serum mixed with agar 1:4. In the middle trough is rabbit anti-N serum.

The question of whether the anomalous protein is parathormone itself must also be considered. In hyperparathyroidism the concentration of the parathormone is increased many times. It is possible that the parathormone as a polypeptide is bound to some serum protein which it might influence to such a degree as to change the electrophoretic mobility or the diffusion rate of the protein component. This is borne out by the fact that after removal of the adenoma the immunoelectrophoreogram reverts to normal.

SUMMARY

1. In 5 patients with hyperparathyroidism, immunoelectrophoresis showed anomalies in the beta-1-globulin area of the serum as compared with normal sera.

2. After operation of the adenoma the protein spectrum reverted to normal.

3. The authors discuss whether the protein normally present in the serum could be influenced by the parathormone to such a degree that the protein is dissociated into 2 compounds with different diffusion rates.

REFERENCES

1. Williams, C. A., Jr., and Grabar, P.: Immunoelectrophoretic Studies on Serum Proteins. The Antigens of Human Serum, J. Immunol. **74**: 158, 1955.
2. Wunderly, Ch.: Die Immunoelektrophorese in Agar Gel, Experientia **13**: 421, 1957.
3. Cleve, H., and Hartmann, F.: Immunoelektrophoretische Untersuchungen in Serum Rheumakranker, Klin. Wchnschr. **35**: 334, 1957.
4. Scheidegger, L.: L'immunoélectrophorèse, Semaine Hôp. Paris **32**: 2119, 1956.
5. Scheiffarth, F., and Götz, H.: Immunoelektrophoretische Studien bei Hypergammaglobulinämie Waldenström, Aerztliche Wchnschr. **13**: 751, 1958.
6. Bronsky, D., Dubin, A., and Kusheser, D. S., De la Huerga, J.: Mucoproteins in Thyroid and Parathyroid Disease: Clinical and Experimental Studies, J. Clin. Endocrinol. **17**: 1922, 1957.

EFFECTS OF AN ACUTE SALT LOAD IN A CASE OF PRIMARY HYPERALDOSTERONISM BEFORE AND NINE MONTHS AFTER SURGICAL CURE

Comparison With Normal Subjects and Patients With Essential Hypertension Before and After Reduction of Their Elevated Pressure With Drug Treatment

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THE sodium retention and potassium excretion capacity of aldosterone is well established, and accounts for most of the electrolyte abnormalities usually found in cases of primary hyperaldosteronism (PHA). It also has been pointed out that in essential hypertension the basal excretion of sodium is increased,[3, 5] and that under an acute salt load the hypertensive patient excretes a higher percentage of the sodium and water load injected, more rapidly than the normal subject. This effect has been correlated with the increased arterial tension[1-5, 7, 9] and also has been found in cases of Cushing's syndrome and hypertension.[6]

A salt load test performed in a case of PHA with marked hypertension might permit a comparison between the pressure factor, leading to increased Na and water excretion, and the hormonal factor tending toward Na retention. The repetition of this test after surgical cure of the PHA, and comparison with the effect observed in essential hypertension before and after reduction of the elevated tension by drug treatment, may permit a comparative study of the influence of both syndromes on hydrosaline metabolism.

The following report shows the result of a sodium loading test performed in a case of PHA before and 9 months after surgical cure by removal of an adrenal adenoma, and its effect on water and electrolyte excretion, changes in plasma, red cells (erythrocytic mass) and extravascular volumes, and distribution of retained Na. These results are compared with those observed with the same test in a control group of normal subjects, and a group with essential hypertension, and in some of the latter after reduction of their elevated tension with hypotensive drugs.

MATERIALS AND METHODS

The case of primary hyperaldosteronism of this experiment has been reported previously,[8] and the principal features are summarized in Tables I and II. The findings in the control group and in patients with treated and untreated hypertension have also been reported.[9]

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TABLE I. CLINICAL DATA ON PATIENT A. M. (26-YEAR-OLD WOMAN)

DATA	PREOPERATIVE OBSERVATION JULY-AUGUST, 1957	POSTOPERATIVE CONTROL APRIL, 1958
Episodes of muscular weakness	+	–
Occipital headache	+	–
Tetanic manifestations	+	–
Polydipsia, polyuria, nicturia	+	–
Diminished sweating	+	–
Range of blood pressure		
Systolic	174-230	140-150
Diastolic	102-122	80-90
Cardiac enlargement	–	–
Edema	–	–
Hypertensive neuroretinopathy	+	–
ECG signs of hypokalemia	+	–
Intravenous pyelography	Normal	

TABLE II. LABORATORY FINDINGS

DATA	PREOPERATIVE OBSERVATION	POSTOPERATIVE CONTROL
Serum K (mEq./L.)	2.7-3.5*	6.2
Serum Na (mEq./L.)	144.4-155.2*	135.5
Serum Cl (mEq./L.)	94-102	103
Serum Ca (mEq./L.)	6	
CO_2 combining power (mEq./L.)	31-35*	21
Blood pH	7.50-7.53*	7.38
Mean urinary volume (ml.)	2,000	1,070
Urinary density (maximum)	1,016 (Concentration test)	1,020 (Spontaneous)
Urine albumin	Traces	–
Urine pH	6.1-8*	5.3
Urine NH_4 (mEq./24 hr.)	56-66	28
U-P K ratio	16.8	7.6
Urine 17-ketosteroids (mg./24 hr.)	5.40-10.20†	
Urine 17-ketogenic corticoids (mg./24 hr.)	6.8†	
Urine pregnanediol (U.U.L./24 hr.)	52.8†	
Saliva Na-K ratio normal controls: 0.65-0.86	0.42-0.56	
B.M.R. (per cent)	–2	
Serum proteins electrophoretic curve	Normal	
Urea clearance (ml.)	76-104‡	72
Endogenous creatinine clearance (ml.)	120-144‡	110
PAH clearance (ml.)	690	
Blood count	Normal	Normal
Blood sugar	Normal	Normal
Sedimentation rate	Normal	Normal

*Range of values in 5 determinations.
†Normal values for this Laboratory.
‡Range of values in 3 determinations.

The loading test was performed while the subjects were maintained on a diet with comparable sodium content. After an overnight fast, the urinary bladder was emptied by means of an indwelling catheter, and this urine discarded. One hour later, a blood sample was withdrawn, the bladder urine collected, and an intravenous infusion of 5 per cent NaCl was started, 500 ml. being injected in one hour. Then another sample of blood was taken and the urine again collected. The urine collection was repeated every half hour for 2 hours, after which a third sample of blood was withdrawn. Three samples of blood (before the test, at the end of the infusion, and two hours after) and 6 of urine (before the test, at the end of the infusion, and then every half hour for 2 hours) were thus obtained. All subjects remained in supine position until completion of the test which took a period of 4 hours, including a control period of one hour before the injection. The blood pressure was taken several times during the test.

Urinary volumes were accurately measured. Sodium and potassium were determined by flame photometer and chloride by the method of Shales and Shales.[10] The changes in plasma volume were measured by determining the plasma specific gravity and plasma protein concentration by the copper sulfate method[11] and applying the formula devised by Rosenbaum.[12, 13] In our experience, this method has proved accurate when properly employed,[14] especially to estimate acute changes in plasma or circulatory volumes. Total erythrocytic mass (red cell volume) was determined by the hematocrit value corrected in accordance with the ratio between values of peripheral and corporal blood[15] and by using the changes in the mean corpuscular hemoglobin concentration as an index of erythrocyte hydration.[16] The changes in extravascular volume produced by the saline injection were determined by simple balance between the injected volume, the urine output during the experimental period, and the variations in plasma and erythrocytic volume.

RESULTS

Table III shows the urinary volumes and the excretion of Na, K, and Cl in the 6 samples of urine collected during the test performed in patient A. M. before and 9 months after the operation. Total excretion of water (1,340 ml. during the 3 hour test) falls to 513 ml. after the operation, a volume similar to the one injected. Total Na excretion falls one half, and Cl excretion falls somewhat less than Na. Potassium excretion, on the other hand, increases remarkably after the operation due to a greater concentration in the smaller urinary volume. The known increase of K excretion produced by the hypertonic Na solution can be appreciated by comparison of the excretion in the control period of one hour previous to the injection (first line in Table III), with that obtained during the first hour of the test.

TABLE III. URINARY VOLUME AND Na, K, AND Cl EXCRETION DURING SALT LOADING TEST PERFORMED BEFORE AND 9 MONTHS AFTER THE OPERATION (500 ML. OF WATER AND 427 MEQ. OF Na AND Cl)

TIME* (A.M.)	URINE VOLUME (ML.)		Na (MEQ.)		K (MEQ.)		Cl (MEQ.)	
	PRE-OPERATIVE	POST-OPERATIVE	PRE-OPERATIVE	POST-OPERATIVE	PRE-OPERATIVE	POST-OPERATIVE	PRE-OPERATIVE	POST-OPERATIVE
7:30								
8:30	157	42	21.2	6.26	5.8†	2.84	23.2	7.94
9:30	840	160	138.3	36.19	9.07	6.83	151.7	41.28
10:00	155	100	30.5	24.62	1.9	4.09	6.6	24.08
10:30	150	70	25.8	17.05	2.0	3.86	23.0	18.52
11:00	128	83	25.7	19.49	2.0	4.60	24.4	20.84
11:30	87	100	18.9	24.36	1.9	5.63	16.1	25.12
Total	1,340	513	239.2	121.71	16.87	25.01	221.8	129.84
Per cent injected load	268	102.6	56	28.5			52	20.2

*The infusion was administered from 8:30 until 9:30 A.M.
†Range of values in normal and hypertensive subjects: 0.84 to 4.45 mEq.

In Fig. 1, these results are compared with those obtained in normal subjects and patients with essential hypertension, in some of the latter after reduction of their elevated tension with hypotensive drugs. In this case of primary hyperaldosteronism the hypertonic saline injection produced increases in water, sodium, and chloride excretion similar to that observed in essential hypertension. When the test was repeated 9 months after the operation, the patient being apparently cured (see Tables I and II), the result obtained resembles that obtained in the group with reduced hypertension.

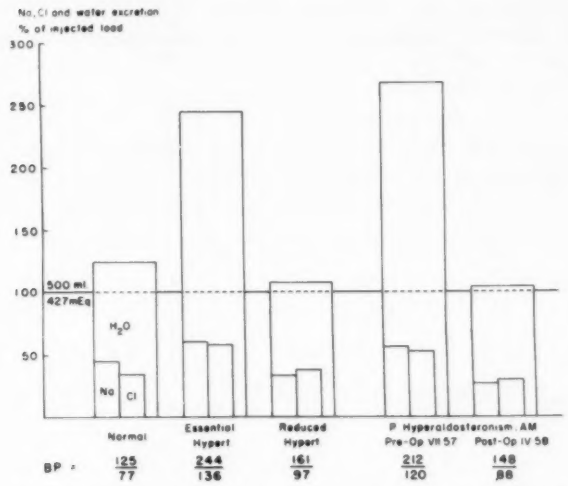

Fig. 1.—Water, sodium, and chloride excretion during 3 hours expressed in percentage of the injected load. One hundred per cent represents 500 ml. of water and 427 mEq. of both Na and Cl. Control group values are means of 6 cases; hypertensive group values are the means of 10 cases; and the reduced hypertensive group values are the means of 3 cases. Blood pressures were recorded several times during the infusion of the salt load, and the values given are the average of each group. In the hypertensive group were 6 patients with cardiac enlargement but without heart or renal failure; in 3 of them a successful lowering of arterial pressure was obtained with a combination of reserpine, pentapyrrolidinium and hydralazine.

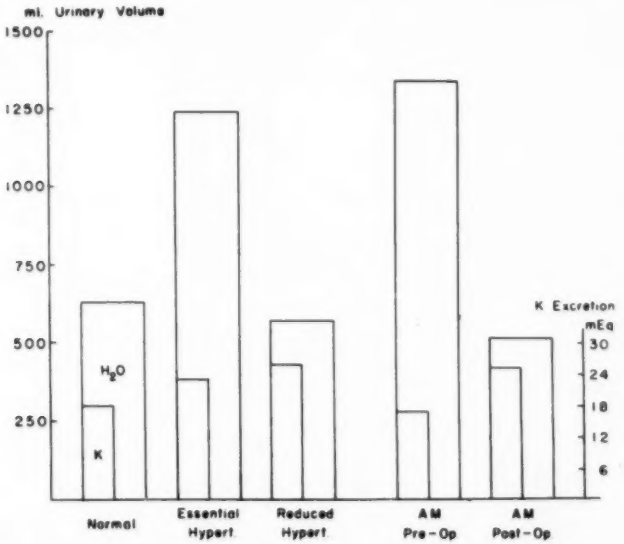

Fig. 2.—Water and potassium excretion in the groups studied and in patient A. M. before and 9 months after the operation.

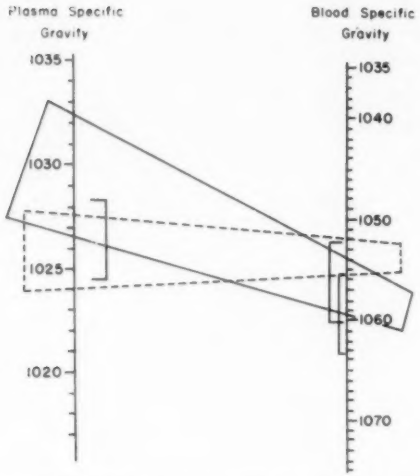

Fig. 3.—Nomograms of Van Slyke in cases with hypertension, reduced hypertension, and in patient A. M. (before and after surgery) determined before saline infusion. The area formed by the continuous line encloses values obtained in essential hypertension and in patient A. M. preoperatively. The dotted line circumscribes those of reduced hypertension and patient A. M. postoperatively. The brackets show the range of normal values. Women have a lower blood specific density.

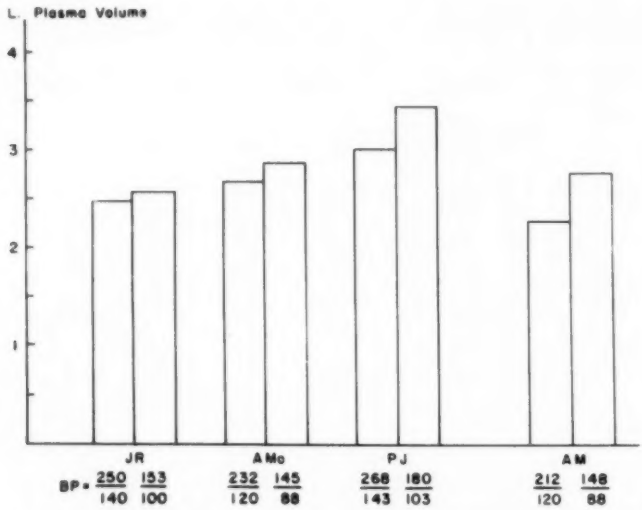

Fig. 4.—Plasma volumes in 3 hypertensive subjects before and during treatment and in patient A. M. before and 9 months after operation. Determinations were made on the blood sample secured before the saline infusion.

Fig. 2 shows the total excretion of urine and potassium in the groups studied and in patient A. M. in a test performed before and 9 months after surgical cure. With the decrease of the urinary output in the postoperative tests to a volume similar to the injected solution, the total K excretion increases from 16.8 to 25.01 mEq., and the mean K concentration from 15 to 50 mEq. per liter. This is quantitatively different from the effect observed in patients with untreated and reduced hypertension. Before the operation potassium excretion is higher in the hypertensive subjects than in patient A. M., and the increase brought about by the reduction of pressure is more marked in patient A. M.

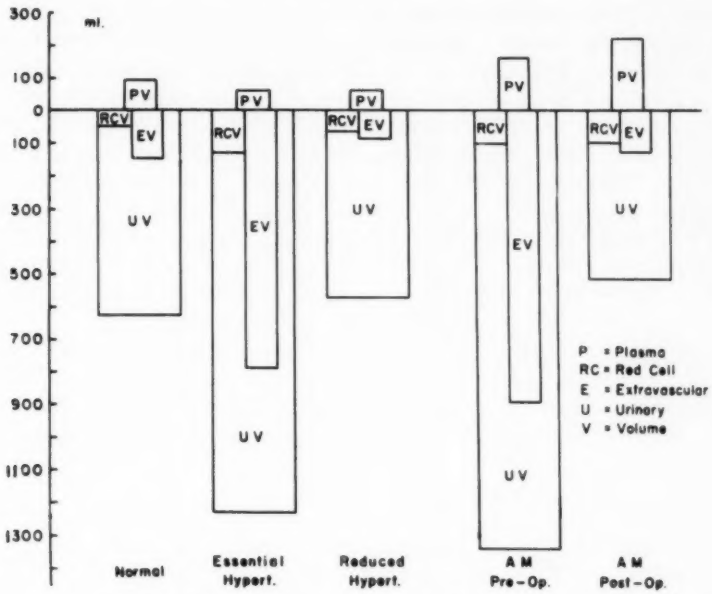

Fig. 5.—Changes in plasma volume, total erythrocytic mass (red cells volume), and extravascular volume at the end of the test (3 hours after the beginning of the infusion). Variations in plus or minus are represented above or below the zero line. Largest area reveals urinary volume during the same period.

Fig. 3, representing the nomograms of Van Slyke, shows the variations in water content of the plasma observed with the decrease of blood pressure. It is evident that the increased plasma density when the pressure is elevated and its subsequent reduction to normal are due to the increase of its water content with the lowering of blood tension. These variations in the water content of the plasma may explain the slight variations of plasma volume with the reduction of blood pressure depicted in the hypertensive subjects and patient A. M. (Fig. 4). We must emphasize that this has been a constant finding in all hypertensive patients where a significant reduction of blood pressure has been obtained; its meaning is discussed in a previous paper.[9]

The injection of the hypertonic saline solution also produces changes in plasma, red cells, and extravascular volumes, probably related to the increased urinary output and the action of osmoregulatory mechanisms. Fig. 5 shows these changes in the groups under study. In the normal controls, the injection of 500 ml. of 5 per cent NaCl produced a water loss of 620 ml. and an increase of 100 ml. in plasma volume. The difference of 220 ml. with the injected water comes from the red cells and the extravascular compartment, with a loss of 50 and 170 ml. of water, respectively. In the hypertensive patients, due to the large urinary volume, the dehydration is more pronounced as is also the water loss in the red cells and the extravascular compartment. In the subjects with reduced hypertension, the changes resemble those observed in the normal group. In patient A. M., the loss of extravascular water before the operation is larger than in the previous groups, due to the large urinary volume and a greater increase in plasma volume.

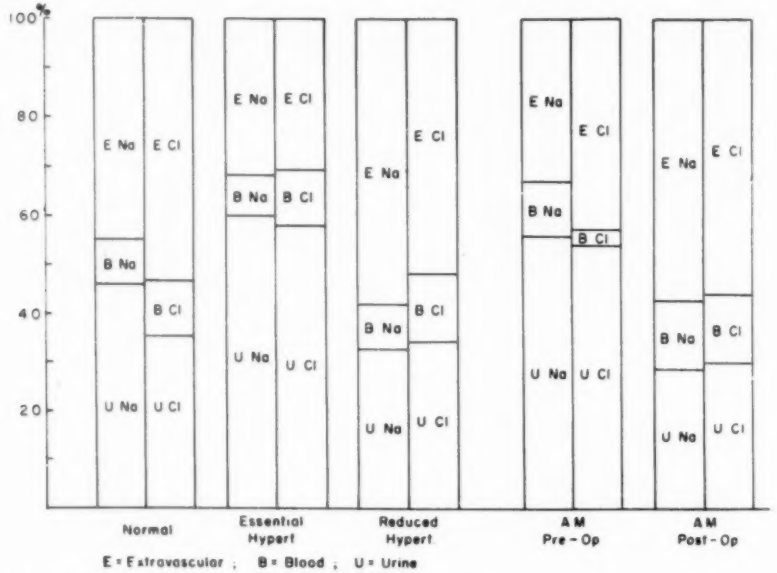

Fig. 6.—Distribution of the injected sodium chloride in the urine, the blood, and extravascular compartment at the end of the test.

Fig. 6 shows the distribution of injected Na in the urine, blood, and extravascular compartment expressed as per cent of the load. As it could be presumed by the amounts excreted, Na retention is greater in subjects with reduced hypertension and in patient A. M. after the operation.

DISCUSSION

In this case of primary hyperaldosteronism, the hypertonic saline injection produced the same increase of water, sodium, and choloride excretion and the

same changes in plasma, red cells, and extravascular volumes that it brings about in essential hypertension. These effects seem to be also related to the elevated tension, since the reduction of pressure through the surgical cure of the case brings about a response similar to that observed in essential hypertension when the blood pressure has been reduced with hypotensive drugs.

Potassium excretion in the control period previous to the injection is naturally very high in patient A. M. before the operation, greater than in any other case in this period. The differences observed in potassium excretion with the loading test in patient A. M. before and after the operation and the hypertensive subjects before and after reduction of the elevated pressure could be explained if we assume that the well-known potassium excretion of hypertonic saline injection might be less marked in a potassium depleted organism, and this becomes evident when, 9 months after the operation, potassium stores have been presumably normalized.

Potassium concentration seems to be independent of the blood pressure and varies inversely with the urinary volume. The greatest concentration and larger excretion are reached in patient A. M. after the operation, with a low pressure and the smallest urinary volume. This is an interesting finding, because as potassium is mainly of tubular origin, it indicates the glomerular origin of the changes that a saline load produces on water, sodium, and chloride excretion in essential hypertension that are not parallel to potassium excretion.

The hypertonic dehydration produced by the saline injection is greater in cases with elevated blood pressure due to the greater excretion of water than sodium as related to the injected load. This increased water excretion in spite of plasma hyperosmolarity might be considered as a consequence of an increased glomerular filtration by the elevated tension.

We believe that the diminished water content of the plasma in all the cases with high blood pressure which becomes normal when the pressure is reduced is also an argument in favor of a mechanic pressure factor which, through variations in glomerular filtration, may be responsible for the effects produced in essential hypertensive subjects by an acute salt load. The increased pressure might increase, although slightly, the glomerular filtration rate with the establishment of a "new steady state" in which more plasma water is excreted with greater concentration of the protein fraction. In laboratory animals it has been shown that this effect is mediated through glomerular ultrafiltration.[17] The small variation necessary to account for this result is probably not always obvious by the clearance techniques employed in human beings.

As a tentative conclusion from the preceding paragraphs, the decisive role of elevated pressure in the increased excretion of water, Na, and Cl produced by saline load in subjects with essential hypertension and in one case of PHA may be pointed out. Before this test, the patient with PHA did not show any appreciable difference from essential hypertensive patients, and no hormonal factor of cortical type was apparently operative in the observed result. This finding is in agreement with that observed in DCA-induced hypertensive rats, which excrete Na more rapidly than normal controls in spite of the sodium retention

effect of this hormone,[18] and with the observation of Hollander and Judson[19] who found that adrenalectomized hypertensive individuals conserve their increased capacity to excrete sodium when given an intravenous saline load.

It must be pointed out also that the characteristics of K excretion, and the changes in water content of the plasma with the reduction of blood pressure, also indicate a mechanical pressure factor, acting probably upon glomerular filtration, which accounts for the increased excretion of water, sodium and chloride produced by a hypertonic saline injection in cases of essential hypertension and this case of PHA.

SUMMARY

A study was made of the effects of the injection of hypertonic saline solution before and 9 months after surgical cure in a case of primary hyperaldosteronism, and these effects were compared with those observed with the same test in normal subjects, and essential hypertensive patients and in some of the latter after reducing their elevated tension with drug treatment. The following conclusions were drawn.

1. The response to the sodium loading test was the same in the case of primary hyperaldosteronism as in the cases of essential hypertension.

2. After surgical cure of the patient with primary hyperaldosteronism, the response was similar to that obtained in cases of drug-reduced hypertension.

3. The role of elevated arterial pressure is apparent, but the influence of a hormonal factor of cortical type is not apparent in the hydrosaline variations produced by the sodium load in the patient with primary hyperaldosteronism and in cases of essential hypertension.

4. The characteristics of potassium excretion, as well as the changes in the water content of the plasma with the reduction of the elevated pressure, indicate that a mechanical pressure factor acting upon glomerular filtration, more than a hormonal factor of cortical type acting on tubular reabsorption, accounts for the increased excretion of water, sodium and chloride produced by a hypertonic saline injection in cases of essential hypertension and this case of primary hyperaldosteronism.

REFERENCES

1. Farnsworth, E. B., and Barker, M. H.: Tubular Reabsorption of Chloride in Hypertensive and in Normal Individuals, Proc. Soc. Exper. Biol. & Med. **52**: 74, 1943.
2. Green, D. M., Wedell, H. G., Wald, M. H., and Learned, B.: The Relation of Water and Sodium Excretion to Blood Pressure in Human Subjects, Circulation **6**: 919, 1952.
3. Green, D. M., and Ellis, E. J.: Sodium Output-Blood Pressure Relationship and Their Modification by Treatment, Circulation **10**: 536, 1954.
4. Hollander, W., and Judson, W. E.: Electrolyte and Water Excretion in Arterial Hypertension. II. Studies in Subjects With Esssential Hypertension After Antihypertensive Drug Treatment, Circulation **17**: 576, 1958.
5. Cottier, P. T., Weller, J. M., and Hoobler, S. W.: Effect of an Intravenous Sodium Chloride Load on Renal Hemodynamics and Electrolyte Excretion in Essential Hypertension, Circulation **17**: 750, 1958.
6. Birchall, R., Tuthill, S. W., Jacobs, W. S., Trautman, W. J., and Frindley, T.: Renal Excretion of Water, Sodium and Chloride: Comparison of the Response of Hypertensive Patients With Those of Normal Subjects, Patients With Specific Adrenal or Pituitary Defects, and Normal Subjects Primed With Various Hormones, Circulation **7**: 258, 1953.

7. Hollander, W., and Chobanian, A. V.: The Renal Excretion of Intravenous 5 Per Cent Sodium Chloride in Subjects With Hypertension, Proc. Clin. Res. **5**: 23, 1957.
8. Ortúzar, R., Thomsen, P., and Gonzalez, J.: Hiperaldosteronismo primario, Rev. méd. Chile **86**: 641, 1958.
9. Croxatto, R., Thomsen, P., González, J., and Ortúzar, R.: Effects of a Salt Overload in Subjects With Normal Arterial Pressure and Essential Hypertension Before and After Reducing Arterial Pressure With Hypotensive Drugs, Jorn. Cardiol. Sud Americanas del Pacifico. Santiago, Dec. 3, 1957. (In press.)
10. Schales, O., and Schales, S. S.: A Simple and Accurate Method for the Determination of Chloride in Biological Fluids, J. Biol. Chem. **140**: 879, 1941.
11. Phillips, R. A., Van Slyke, D. D., Dole, V. P., Kendall, E., Jr., Hamilton, P. B., and Archibald, R. M.: Copper Sulfate Method for Measuring Specific Gravities of Whole Blood and Plasma, New York, 1945, Josiah Macy, Jr., Foundation.
12. Weisberg, H. F.: Water and Electrolyte Metabolism, Editorial Artécnica, Buenos Aires, 1954, pp. 178-179.
13. Rosenbaum, J. D.: Water Balance and Electrolytes, Acidosis and Alkalosis *in:* Ham, T. H.: A Syllabus of Laboratory Examinations in Clinical Diagnosis, Cambridge, 1950, Harvard University Press, pp. 425-452.
14. Croxatto, R., Salvestrini, H., Badía, W., and Rosati, S.: Contribution to the Study of the Modifications That Proteins, Hematocrit, Hemoglobin, and Circulatory Volume Undergo in Relation to Surgery, Arch. Soc. cirujanos hosp. Num. Extraordinario IX Congreso Chileno de Cirugía, 1947, p. 92.
15. Zak, G. A., and Earle, D. P.: Blood Volume and Body Water in Normal Hospital Subjects, J. Lab. & Clin. Med. **49**: 504, 1957.
16. Streeten, D. H. P., and Thorn, C. W.: Use of Changes in the Mean Corpuscular Hemoglobin Concentration as an Index of Erythrocyte Hydration, J. Lab. & Clin. Med. **49**: 661, 1957.
17. Selkurt, E. E.: Effect of Pulse Pressure and Mean Arterial Pressure Modification on Renal Hemodynamics and Electrolyte and Water Excretion, Circulation **4**: 541, 1951.
18. Conn, J. W.: Adrenal Factors in Hypertension, Circulation **17**: 743, 1958.
19. Hollander, W., and Judson, W. E.: Electrolyte and Water Excretion in Arterial Hypertension. I. Studies in Non Medically Treated Subjects With Essential Hypertension, J. Clin. Invest. **36**: 1460, 1957.

EFFECT OF ERYTHROPOIETIN ON THE MITOTIC RATE OF ERYTHROBLASTS IN BONE MARROW CULTURES

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With the Technical Assistance of Edith Menzer

HUMORAL control of erythropoiesis has been under active study in recent years and the growing volume of literature on the subject has been extensively reviewed by Brecher and Stohlman.[1] The circulating anoxic factor, in particular, has received much attention from several groups of investigators. This factor, which mediates the anoxic stimulus to erythropoiesis, was originally named hématopoiétine by Carnot and Deflandre.[2] The more specific term erythropoietin[3] has now gained more general acceptance.

In vivo techniques for the demonstration and estimation of erythropoietin activity are employed by most workers. Using an in vitro technique developed in this laboratory, we were able to show a significant increase in the proliferative activity of human bone marrow erythroid cells when grown with sera from patients with severe anemia or with cyanotic congenital heart disease with polycythemia.[4] The in vitro approach offered an advantage because it allowed the direct study of changes in an isolated bone marrow cell population. The method as published, however, was too crude to be used for the quantitative assay of erythropoietin activity.

In the search for a finer index of the proliferative activity of erythropoietic tissue in vitro, our method was modified by the incorporation of colchicine into the culture medium, as it was applied to bone marrow cultures by Astaldi and Mauri.[5, 6] In the presence of colchicine, mitoses are arrested at prometaphase. As incubation continues, arrested mitoses accumulate in the culture, so that the number of mitoses found after a given period of incubation is an expression of the number of cells which have entered mitosis during this period.

The present report deals with the estimation of erythropoietin activity of anemic rabbit serum in bone marrow cultures containing colchicine. With this technique we have been able to confirm our previous results and make some additional observations on the mode of action of erythropoietin.

MATERIAL AND METHODS

The method described in detail elsewhere[4] was followed. Briefly, the method consists of incubating cell suspensions from bone marrow aspirates in homologous plasma clots. One-tenth milliliter aliquots of the washed bone marrow cells, suspended in Gey's solution con-

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taining thrombin, are blown onto a layer of plasma at the bottom of a series of culture tubes. A firm clot forms almost immediately. One milliliter of culture medium is then added, consisting of 3 parts of a serum to be tested and 1 part of Gey's solution. The cultures are then incubated at 37° C. in a roller apparatus. At the end of incubation, imprint smears are made from the intact pellicle of clotted plasma and stained with May-Grünwald-Giemsa for differential counts.

In the present work, the original method was used with the following modifications: bone marrow, serum, and plasma were obtained from rabbits, colchicine, to a final concentration of 1:500,000, was added to the culture medium, and total nucleated cell counts were omitted.

Rabbits were made anemic by bleeding. Thirty to forty milliliters of blood was withdrawn daily. The animals were exsanguinated when the hemoglobin had reached a level of 4 to 5 Gm. per cent. Reticulocyte counts at this level of hemoglobin were 20 to 30 per cent.* Normal serum was obtained from rabbits whose hemoglobin level was above 11 Gm. per cent. Normal and anemic sera, respectively, were pooled and frozen.

Bone marrow was obtained by aspiration from the tibiae and femurs of nonanemic animals, except when otherwise indicated. Care was taken not to aspirate quantities of material of more than 0.5 ml. from each bone in order to minimize contamination with peripheral blood. Aspirates from more than one bone were sometimes pooled. Material was discarded unless it contained, after washings, at least 50,000 nucleated cells per cubic millimeter.

One part by volume of bovine thrombin (Upjohn), diluted in Gey's solution to 200 units per milliliter, was added to 2 parts of the sedimented cells after the second washing.

Imprint smears were prepared by carrying the clot in zigzag-fashion under slight pressure over the slide. Smears prepared in this manner were found more suitable for counting. An alternative method, in which the cells were centrifuged and smeared following lysis of the clot with streptokinase, resulted in an uneven distribution of the cells, thereby introducing an error in counting.

The number of mitotic figures per 1,000 mitotable erythroid cells (i.e., proerythroblasts, early and intermediate normoblasts) was determined. In each culture 2,000 to 3,000 cells were counted by at least 2 observers.

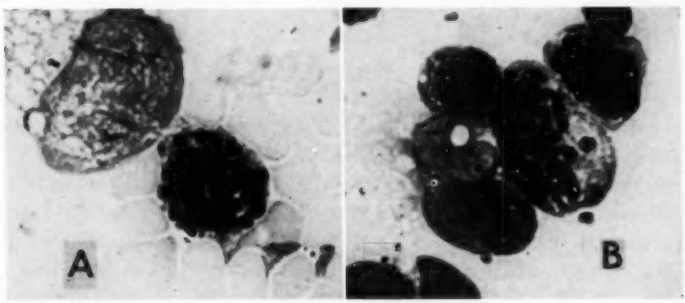


Fig. 1.—*A*, Erythroblast arrested at prometaphase by colchicine. Pyknotic chromosomes scattered throughout cell. *B*, Erythroblast in mitosis (right upper), polychromatic normoblast in interphase (left upper) and myelocyte in mitosis (center).

RESULTS

Mitotic figures were present in conspicuously large numbers in cultures over 5 hours old. They showed the morphological abnormalities typical of erythroid cells arrested in prometaphase by colchicine. The chromosomes

*In rabbits made anemic by phenylhydrazine, reticulocyte counts were 80 to 90 per cent at analogous hemoglobin levels. However, incubation with sera obtained from rabbits previously treated with phenylhydrazine resulted in a considerable decrease in the number of cells in cultures. Because of this toxic effect the use of phenylhydrazine was discontinued.

TABLE I. MITOTIC RATE AT VARIOUS DILUTIONS OF ANEMIC SERUM IN NORMAL SERUM INCUBATED FOR 10 HOURS

SERUM (PER CENT)		MITOSES PER 1,000 CELLS
ANEMIC	NORMAL	
100	0	303
75	25	271
50	50	214
25	75	178
0	100	122

appeared thickened and pyknotic, and stained deep blue with May-Grünwald-Giemsa. In older mitoses the chromosomes tended to be diffusely scattered throughout the cell or to fuse into amorphous chromatin masses. Because of their striking appearance, "colchicine mitoses" in erythroid cells can be distinguished at first glance from cells in interphase as well as from mitoses in other types of cells (Fig. 1).

The number of mitoses increased with the hours of incubation in a more or less linear fashion. In cultures with anemic serum this increase was more marked and more rapid. The difference between normal and anemic serum became apparent after 5 hours of incubation and was most marked between 9 and 12 hours.

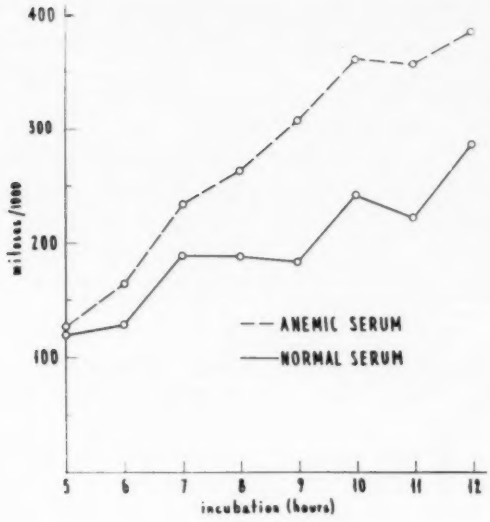
Typical experiments, in which parallel cultures of the same bone marrow were set up with anemic and normal serum, are represented graphically in Fig. 2. It will be seen that, although the same trend of an increased mitotic rate in anemic serum as compared with its normal control is reproduced in each experiment, the shape and slope of curves in individual experiments vary considerably.

When serial dilutions of anemic serum in normal serum were made, it was observed that the number of mitoses decreased with increasing dilution (Table I).

Bone marrow was obtained from a rabbit whose hemoglobin level was reduced to 7 Gm. per cent by a single bleeding the previous day. The marrow, which showed a high degree of erythroid hyperplasia, was washed and incubated with normal and anemic serum. Following the initial sampling at 5 hours, the mitotic rate was observed to rise rapidly, reaching maximum values of 400 to 500 mitoses per 1,000 cells after 9 to 11 hours of incubation with either anemic or normal serum (Table II). Bone marrow from animals with varying levels of hemoglobin was incubated, after washing, with the same normal serum; it was found that for a given period of incubation the number of mitoses was higher, the lower the hemoglobin level of the marrow donor animal (Table III).

TABLE II. MITOTIC RATE OF BONE MARROW FROM ANEMIC ANIMAL INCUBATED WITH NORMAL AND ANEMIC SERUM

INCUBATION (HOURS)	MITOSES PER 1,000 CELLS	
	NORMAL SERUM	ANEMIC SERUM
5	160	204
7	387	394
9	480	430
11	455	460



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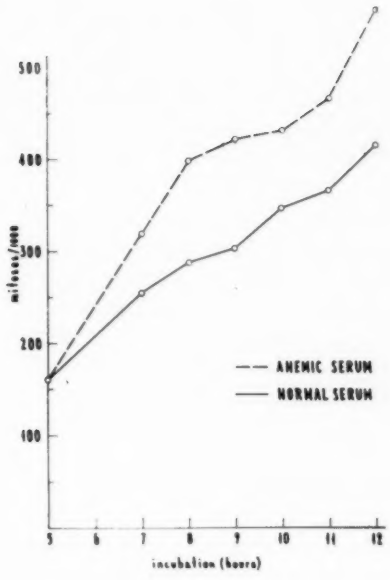

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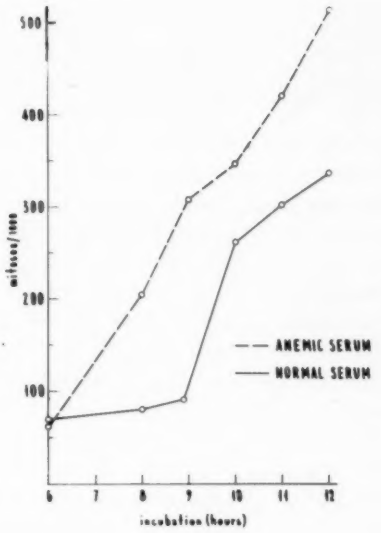

C.

Fig. 2.—Parallel cultures of the same bone marrow with normal and anemic serum. Abscissae : incubation time in hours. Ordinates: mitotic figures per 1,000 mitotable erythroid cells.

TABLE III. MITOTIC RATE AT VARYING LEVELS OF HEMOGLOBIN OF MARROW DONOR INCUBATED FOR 10 HOURS IN NORMAL SERUM

MARROW DONOR (HGB., GM. PER CENT)	MITOSES PER 1,000 CELLS
10.8	316
6.6	354
4.6	556

Mitotic rates for marrow from anemic animals in normal serum (Tables II and III) were considerably higher than those found for normal bone marrow under similar conditions. They were at least as high as the highest rates obtained in cultures of normal bone marrow in anemic serum (Fig. 2).

DISCUSSION

Our data, in addition to providing further evidence for the presence of an erythropoietin in anemic serum, permit of certain conclusions regarding its mode of action.

Erythropoietin is believed to act by stimulating erythroblasts in the bone marrow to increased mitotic activity. This mechanism of action has been inferred from observations concerning the effect of erythropoietin on the peripheral blood. Criteria commonly used for the evaluation of such an effect are polycythemia, reticulocytosis, or an increased rate of incorporation of Fe^{59} into erythrocytes. Interpretation of these phenomena, however, does not necessarily exclude an alternative mode of action of erythropoietin, for example, acceleration of the rate of maturation or of the release of erythroid cells into the circulation. In our experiments the mitotic activity of an isolated population of erythroblasts was studied and found to be increased in the presence of anemic serum. This observation provides direct evidence for a mitosis-stimulating effects of erythropoietin at the bone marrow cellular level.

The intimate contact and interaction between erythropoietin and bone marrow erythroid cells is further emphasized by the high mitotic rates obtained when washed bone marrow cells from anemic animals were incubated with normal serum. These high mitotic rates, comparable to the highest rates found on incubation of bone marrow from nonanemic animals with anemic serum, were in all likelihood due to the presence, in vivo, of erythropoietin within the cells of the anemic animal or closely attached to them. It would seem that erythropoietin is taken up by erythroblasts and then exerts its mitosis-stimulating effect from within the cells.

Bone marrow from anemic animals was hyperactive as a result of erythropoietic stimulation in vivo. Transfer to the cultures of the in vivo stimulus rather than of mitoses actually present in vivo accounted for the high mitotic activity in vitro, since practically all mitotic figures found in the cultures had the typical appearance of pathological prometaphases arrested by colchicine. This was true for the entire period of incubation, from the initial sampling at 5 hours throughout the subsequent hours of incubation during which the number

of mitoses increased threefold. With an estimated mitotic time of 45 minutes for human erythroid cells,[7] all these mitoses must have occurred and arrested during incubation.

Differences in the response of bone marrow from individual animals probably account, at least in part, for the lack of complete uniformity in results. With the same bone marrow, a higher mitotic rate in the presence of anemic serum was constantly observed. However, using different "normal" bone marrows, it was not possible to construct a reproducible standard curve for erythropoietin activity in relation to the hemoglobin level of the serum donor animal. As bone marrow had to be freshly obtained for each experiment, it remained the only component of the system which could not be standardized.

SUMMARY

Bone marrow cell suspensions obtained from rabbits were grown in homologous plasma clots in the presence of serum from normal rabbits and from rabbits made anemic by bleeding. Colchicine was incorporated into the culture medium, and the number of mitoses arrested by colchicine and found in the culture at the end of incubation was used as an index of mitotic activity.

The number of mitoses increased with the hours of incubation. The increase was higher in cultures with anemic serum.

The number of mitoses decreased with progressive dilution of anemic serum with normal serum.

When bone marrow from an anemic animal was incubated with normal serum, high mitotic rates were obtained. These were comparable to the highest rates found on incubation of normal bone marrow with anemic serum.

It is concluded that erythropoietin is present in erythroid cells. It acts by stimulating the cells to mitotic division.

REFERENCES

1. Brecher, G., and Stohlman, F.: Humoral Factors in Erythropoiesis. Progress in Hematology, New York, 1959, Tocantins, Grune & Stratton.
2. Carnot, P., and Deflandre, C.: Sur l'activité hématopoiétique du sérum au cours de la régéneration du sang, Compt. rend. Acad. sc. **143**: 384, 1906.
3. Bonsdorff, E., and Jalavisto, E.: A Humoral Mechanism in Anoxic Erythrocytosis, Acta physiol. scandinav. **16**: 150, 1948.
4. Matoth, Y., Biezunski, N., and Szabo, G.: Effect of Sera From Patients With Anoxia on the Proliferative Activity of Human Erythropoietic Tissue In Vitro, J. LAB. & CLIN. MED. **51**: 420, 1958.
5. Astaldi, G., and Mauri, C.: La vulutazione dell'attività proliferativa delle cellule midollari. Studio di un "test statmocinetico," Haematologica **33**: 583, 1949.
6. Astaldi, G., and Mauri, C.: New Criteria for the Evaluation of the Bone-Marrow Cells Mitotic Activity, Sang **21**: 378, 1950.
7. Patt, H. M.: A Consideration of Myeloid-Erythroid Balance in Man, Blood **12**: 777, 1957.

INEFFICIENT HEPATIC EXTRACTION OF COLLOIDAL GOLD: RESULTING INACCURACIES IN DETERMINATION OF HEPATIC BLOOD FLOW

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FOLLOWING the description in 1944 by Warren and Brannon[1] of the technique of hepatic vein catheterization, Bradley and associates[2] adopted this procedure to measure the hepatic extraction of Bromsulphalein (BSP). If the peripheral blood level of BSP is kept constant by continuous infusion, liver blood flow can be estimated by the Fick principle. The method has been extensively discussed[3-8] and is certainly subject to error, principally due to imperfect sampling of mixed venous hepatic blood and to extrasplanchnic removal of the dye. In the presence of gross liver disease, the hepatic BSP extraction may be too low for the method to be applicable, and the procedure is tedious, even for healthy patients.

Intravenously injected colloidal particles of a size such that they do not cross the capillary barrier are removed by the reticuloendothelial cells lining the blood vessels, principally by the Kupffer cells of the liver and the reticular cells of the spleen.[9-13] Provided that the quantity of colloid injected is below a critical amount, the rate of blood clearance of colloid, an exponential function of time, is constant and is independent of the size of the dose. In this circumstance extrasplanchnic uptake of the colloid is negligible, and the liver removes with high efficiency nearly all of the colloid present in the blood passing through it; hence the rate of colloid disappearance from the peripheral blood is essentially a parameter of hepatic blood flow.

In 1953, Dobson and associates[14] used the clearance of colloidal chromic phosphate (labeled with radiophosphorus) to measure liver blood flow; other workers have used colloidal radiogold[15-18] and radioiodinated denatured serum albumin.[13, 19] Published results with these colloids have been consistent but tend to be slightly lower than those obtained with BSP, presumably because the extraction of the colloid is not complete in one passage through the liver.

We have estimated the liver blood flow of 45 patients with colloidal gold because of its ready availability and its easy detection, if required, by external counting. Since the trend of results was considerably lower than expected, direct measurements of hepatic extraction were made, by the technique of hepatic vein catheterization.

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METHODS

Subjects.—The 10 normal subjects were hospital patients convalescing from illnesses not known to affect the liver. Serum alkaline phosphatase, thymol flocculation, zinc sulfate turbidity, and Bromsulphalein retention were normal in each case. Hepatic disease was established by the usual clinical and laboratory methods, the diagnosis being confirmed by liver biopsy in most cases. Prior to any procedure, each subject had been at bed rest for at least 12 hours and fasting for not less than 4 hours.

Colloidal Gold (Au^{198}).—The gold colloid was obtained from the Radiochemical Centre, Amersham, England; this was also the source of the radiogold used by Vetter and co-workers,[15, 16] Krook,[17] and Riddell and associates.[18] It was supplied as a gelatin stabilized suspension containing 5 to 15 μg per milliliter; the average particle size was determined on each batch, but it was stated that electron microscopy revealed that the majority lie between 20 and 25 mμ, with a small percentage as small as 5 mμ and as large as 60 mμ.

Determination of Hepatic Blood Flow by the Radiogold Method.—A Cournand arterial needle was inserted into the brachial artery and blood was withdrawn at 1, 2, 3, 5, 7, 12, and 18 minutes after the intravenous injection of about 15 μc of radiogold (in our earlier experiments blood samples were collected up to 60 minutes). After hemolysis with saponin, the radioactivity of each sample was determined with a scintillation counter employing a thallium-activated sodium iodide crystal.

When these results were plotted on semilogarithmic paper as a function of time, a multicomponent exponential curve was obtained, the initial portion of which approximated the straight line

$$C_t = C_o e^{-kt}$$

where C_t is the concentration at any time t, C_o is the initial concentration, and k is a constant, termed the disappearance constant.[9]

The disappearance constant can be related to the half-time of disappearance by the expression $k = \frac{\log_e 2}{t\frac{1}{2}}$, and the estimated liver blood flow (EHBF) was obtained by multiplying the blood volume by k, the blood volume being assumed to equal 70 ml. per kilogram. In 1956, Dobson and his associates[20] pointed out that the proper volume to use in this calculation is the extrasplanchnic blood volume and not the total blood volume, since the event which leads to the removal of a particle from possible detection in the peripheral blood occurs when the blood leaves the aorta destined for the liver, rather than when the particle is actually phagocytosed by a Kupffer cell. However, since in all other published results the total blood volume has been used, we have done the same.

We were unable accurately to separate graphically the disappearance curve into a number of simple exponential curves. Therefore, like Dobson and associates[14] and Biozzi and co-workers,[19] we drew the line of best fit through the earlier points on the graph, and used this line to calculate the half-time of disappearance.

Like most other workers[13, 15, 16-19, 21] we did not employ simultaneous Evans blue dye administration to eliminate errors due to slow mixing of the colloidal gold with the general circulation. In most patients, including those with liver disease, this correction is said to be negligible.[22]

Measurement of Hepatic Radiogold Extraction.—Each of the 16 subjects studied in this manner was given 0.1 Gm. of pentobarbital 1 hour before the procedure. A Cournand arterial needle was placed in the brachial artery of one arm and, under fluoroscopic control, a cardiac catheter was inserted into the right hepatic vein via the medium cubital vein. Measurements of free and occluded hepatic vein pressure were taken using a strain gauge manometer connected to a Sanborn Cardiette recorder; in each case a convincing gradient was obtained. Throughout the experiment, the tip of the catheter was maintained in the center of the right hepatic lobe, its position being checked fluoroscopically at the conclusion of the observation. The catheter was kept patent with a very slow drip of isotonic saline containing 500 units of heparin in 500 ml. Prior to sampling from the hepatic vein, the catheter was cleared by withdrawing at least 5 ml. of blood and discarding it.

TABLE I. ESTIMATED HEPATIC BLOOD FLOW: RESULTS OBTAINED WITH COLLOIDAL GOLD

PATIENT	DIAGNOSIS	SEX	AGE	WEIGHT (KG.)	BLOOD VOLUME (ML.)	$t_{1/2}$ (MIN.)	K (PER MIN.)	EHBF (ML. PER MIN.)
	Patients Without Liver Disease							
1. C. C.	Gastric ulcer	M	32	65.5	4,600	5.6	0.12	560
2. T. F.	Diverticulosis	M	64	51.8	3,600	2.5	0.28	1,000
3. J. M.	Normal	M	34	65.9	4,600	3.4	0.21	970
4. J. D.	Diabetes	M	60	59.5	4,200	3.7	0.19	780
5. F. G.	Nephritis	M	31	76.4	5,300	4.9	0.14	760
6. G. B.	Bronchitis	M	49	59.8	4,200	5.5	0.13	550
7. L. B.	Postgastrectomy syndrome	F	37	44.4	3,100	4.0	0.17	530
8. B. W.	Gastric erosions	F	30	51.0	3,600	2.3	0.30	1,100
9. L. N.	Postgastrectomy syndrome	F	46	48.2	3,400	3.0	0.23	780
10. O. R.	Gastritis	F	32	48.6	3,400	4.4	0.16	570
	Patients With Cirrhosis							
11. R. S.	Cirrhosis	M	40	69.0	4,800	6.5	0.11	530
12. H. T.	Cirrhosis	M	51	46.8	3,300	5.1*	0.14	460
						5.8†	0.12	400
13. W. W.	Cirrhosis	M	49	56.8	4,000	5.6*	0.12	480
						5.1†	0.14	560
14. H. G.	Cirrhosis	M	46	66.8	4,700	18.0	0.038	180
15. S. G.	Cirrhosis	M	45	65.9	4,600	12.9	0.054	250
16. A. S.	Cirrhosis	M	38	90.1	6,300	5.8	0.12	760
17. C. S.	Cirrhosis	M	55	86.4	6,000	8.7	0.080	480
18. W. B.	Cirrhosis	M	41	73.6	5,100	5.4	0.13	660
19. A. B.	Cirrhosis	M	49	76.4	5,300	5.0	0.14	750
20. I. K.	Cirrhosis	M	56	88.2	6,200	7.1	0.098	600
21. R. H.	Cirrhosis	M	49	69.2	4,800	8.4	0.082	400
22. J. G.	Cirrhosis	M	47	79.5	5,600	4.1	0.17	950
23. J. A.	Cirrhosis	M	35	89.0	6,200	9.8	0.071	440
24. J. H. U.	Cirrhosis	M	17	68.6	4,800	2.9*	0.24	1,200
						2.7†	0.26	1,200
25. R. C.	Hemochromatosis	M	55	45.2	3,200	4.8	0.14	450
26. J. H.	Hemochromatosis	M	56	50.4	3,500	5.7	0.12	420
27. J. J.	Cirrhosis	F	24	62.3	4,400	6.0	0.12	530
28. S. D.	Cirrhosis	F	30	63.6	4,400	3.1*	0.22	970
						2.6†	0.27	1,200
29. G. H.	Cirrhosis	F	32	53.2	3,700	5.7	0.12	440
30. A. E.	Cirrhosis	F	21	59.5	4,200	6.1	0.11	460
31. M. F.	Cirrhosis	F	43	51.0	3,600	5.9	0.12	430
32. S. P.	Cirrhosis	F	56	52.3	3,700	1.8	0.38	1,400
33. S. P. I.	Cirrhosis	F	29	58.6	4,100	4.1	0.17	700
34. E. T.	Cirrhosis	F	67	61.4	4,300	13.5	0.051	220
35. H. R.	Cirrhosis	F	12	39.5	2,800	4.3	0.16	450
	Other Patients With Liver Dysfunction							
36. J. W.	Extrahepatic portal hypertension	M	32	66.8	4,600	7.0	0.099	460
37. J. C.	Extrahepatic portal hypertension	M	27	68.5	4,800	7.8	0.089	430
38. R. D.	Extrahepatic portal hypertension	M	13	46.4	3,200	3.4	0.20	650
39. E. W.	Extrahepatic portal hypertension	F	42	56.0	3,900	6.2	0.11	430
40. R. P.	Hepatitis	M	25	54.0	3,800	5.6	0.12	460
41. R. H. A.	Fatty liver	M	63	78.2	5,500	4.4*	0.16	880
						4.6†	0.15	830
42. A. T.	Hepatitis	F	68	70.2	4,900	4.9	0.14	690
43. A. L.	Chlorpromazine jaundice	M	48	66.8	4,700	4.7	0.15	700
44. O. F.	Cardiac failure	F	36	50.0	3,500	4.2	0.16	560
45. E. W. O.	Myelofibrosis	F	62	58.6	4,100	4.6	0.15	620

Mean EHBF in 10 normal patients: 760 ml. per minute; standard deviation: 220 ml. per minute. Mean EHBF in 25 cirrhotic patients: 620 ml. per minute; standard deviation: 320 ml. per minute. Mean EHBF in complete group of 45 patients: 650 ml. per minute; standard deviation: 290 ml. per minute.

*A small quantity of radiogold was used.

†Procedure was repeated with a larger quantity of radiogold.

Radiogold was injected intravenously and serial 5 ml. blood samples were taken into heparinized syringes from both the brachial artery and the hepatic vein, allowance being made for the delay in the appearance of the hepatic vein blood at the outer end of the catheter. The samples were taken at the same time intervals as in the experiments already described.

The colloidal gold extraction by the liver was calculated as the difference between brachial artery and hepatic vein blood radioactivity expressed as a percentage of the former.

In 5 subjects a second dose of radiogold was given after an interval of about 45 minutes, and the procedure repeated. In these patients the first dose of colloid was between 3 and 20 μg and the second dose between 50 and 150 μg.

Measurement of Hepatic BSP Extraction and Blood Flow.—Bromsulphalein extraction was determined in 15 of the 16 patients submitted to hepatic vein catheterization. An infusion of BSP in isotonic saline was given intravenously at a constant rate, and the extractions of BSP and colloidal gold were measured at approximately the same time and, in most cases, on the same blood samples.

In 10 subjects EHBF was calculated from the observed extraction and the infusion rate according to the standard method.[2] Only those cases were acceptable in which the BSP extraction was greater than 10 per cent if the peripheral level was in excess of 1 mg. per cent, and greater than 15 per cent when the peripheral level was less than 1 mg. per cent. Allowance was made for changing BSP levels in the peripheral blood, provided that the rate of change was not greater than 0.0002 mg. per milliliter per minute; for this calculation plasma volume was assumed to be 40 ml. per kilogram.

Plasma BSP concentrations were read in a Beckman spectrophotometer at 580 and 620 mμ according to the method described by Gaebler.[23]

Hematocrit determinations were made in duplicate on oxalated arterial blood spun in Wintrobe tubes at 3,000 r.p.m. for 30 minutes, and a correction factor (0.9) applied for whole body hematocrit and trapped plasma.

TABLE II. ESTIMATED HEPATIC BLOOD FLOW: RESULTS REPORTED FOR NORMAL INDIVIDUALS AND FOR CIRRHOTIC PATIENTS

METHOD	YEAR	AUTHORS	REFERENCE	NORMAL SUBJECTS		CIRRHOTIC PATIENTS	
				NUMBER	EHBF	NUMBER	EHBF
BSP	1947	Myers	3	9	1,390		
BSP	1950	Sherlock et al.	4	32	1,400		
BSP	1952	Bradley et al.	5	91	1,530	39	1,090
BSP	1952	Wilkins et al.	24	21	1,380		
BSP	1953	Bradley et al.	25			10	1,266
BSP	1953	Shackman et al.	26	18	1,260		
BSP	1955	Edwards	27			7	1,380
BSP	1958	Redeker et al.	28			10	1,493
Urea	1947	Myers	3	10	1,780		
Chromic phosphate	1953	Dobson et al.	14	29	1,600		
Chromic phosphate	1955	Nardi	21			9	1,800
Denatured albumin	1958	Biozzi et al.	19	5	1,060	6	600
Gold	1954	Vetter et al.	15	25	1,310	12	525
Gold	1956	Vetter et al.	16	15*	770		
Gold	1956	Krook	17	30	731	33	437
Gold	1957	Riddell et al.	18	19	680†		
Gold	1958	Fauvert et al.	29	22	830‡	39	460‡
Gold	1959	Present authors		10	760	25	620

*This group includes some subjects with minor hepatic dysfunction.

†This figure is the one calculated from the blood disappearance curves of gold colloid (see text).

‡This figure is approximate, having been derived from the reported mean disappearance constant and an assumed mean blood volume.

RESULTS

Our mean result for hepatic blood flow in the whole group of 45 subjects was 650 ml. per minute. The figure for the normal subjects was 760 ml. per minute, and for the 25 cirrhotic patients 620 ml. per minute (Table I).

By comparison, the widely accepted values obtained by Bradley and associates,[5] using the BSP method, were 1,530 ml. per minute for normals and 1,090 ml. per minute for a group of 39 cirrhotic patients. A summary of the results obtained by several groups of investigators using a number of methods is set out in Table II.

TABLE III. HEPATIC EXTRACTION OF COLLOIDAL GOLD AND BSP, AND A COMPARISON OF THE DERIVED VALUES FOR EHBF

PATIENTS		HEPATIC COLLOIDAL GOLD EXTRACTION (%) 2 MIN.	3 MIN.	5 MIN.	7 MIN.	12 MIN.	MEAN EARLY GOLD EXTRACTION (%)	MEAN BSP EXTRACTION (%)	EHBF (Au)* (ML./MIN.)	EHBF (Au) (CORRECTED)† (ML./MIN.)	EHBF (BSP)‡ (ML./MIN.)
Normal Subjects											
1. C. C.		31	29	26	23	23	27	55	560	2,100	1,900
3. J. M.		79	79	58	65	—	70	77	970	1,400	1,300
8. B. W.		59	54	54	43	17	55	27	1,100	2,000	2,300
9. L. N.		37	21	36	27	0	31	27	780	2,500	1,600
Patients With Liver Disease											
11. R. S.		30	31	30	32	14	31	11	530	1,700	1,200
12. H. T.	(1)	14	15	10	—	—	13	13	460	3,500	2,200
	(2)	28	24	23	18	10	25		400	1,600	
13. W. W.	(1)	12	18	23	27	—	20	7	480	2,400	—
	(2)	18	22	8	24	8	18	6	560	3,100	—
22. J. G.		24	19	36	34	—	28	10	950	3,400	1,300
23. J. A.		23	23	18	12	15	19	15	440	2,300	2,300
24. J. H. U.	(1)	19	14	48	—	—	27	11	1,200	4,400	—
	(2)	33	45	40	35	0	39	4	1,200	3,100	—
27. J. J.		8	9	7	0	0	8	Negligible	530	6,600	—
28. S. D.	(1)	31	29	31	21	—	31	8	970	3,100	—
	(2)	44	39	38	30	11	40	5	1,200	3,000	—
29. G. H.		21	14	12	0	0	15	22	440	2,900	1,500
30. A. E.		15	—	13	13	6	14	Negligible	460	3,300	—
36. J. W.		8	14	13	18	0	13	—	460	3,500	—
41. R. H. A.	(1)	53	45	49	39	—	50	63	880	1,800	1,300
	(2)	53	50	47	39	24	50		830	1,700	

*EHBF (Au) = Estimated hepatic blood flow using the blood disappearance of colloidal gold.

†EHBF (Au) (Corrected) = $\frac{\text{EHBF (Au)}}{\text{Mean early gold extraction}}$.

‡EHBF (BSP) = Estimated hepatic blood flow using BSP.

Hepatic Extraction of Colloidal Gold.—It was found that the extraction of gold by the liver varied greatly from subject to subject, but in no instance did it approach 100 per cent.

The initial mean extraction, which corresponds to the early "straight line" portion of the disappearance curve, was 27, 70, 55, and 31 per cent in the 4 normal patients, and varied between 8 and 50 per cent with a mean of 23 per cent, in the subjects with liver disease (Table III).

In the latter group, no consistent correlation was found between the colloidal gold extraction and the patient's clinical state, biochemical findings, liver

pathology as determined from biopsy, or portal pressure (measured in every case by the hepatic vein wedged pressure). The extraction of Bromsulphalein was determined in all but one of the subjects; there was a general trend for low gold extractions to be associated with low BSP extractions.

Correlation With Hepatic Blood Flow by BSP Method.—In 10 cases there were simultaneous estimates of hepatic blood flow using colloidal gold and BSP. For this to be possible the extraction of the BSP had to be adequate and its peripheral blood level constant (Table IV). In Subject R. S. the EHBF was determined with a high peripheral Bromsulphalein level, so the result may be too high.

In Table III, determinations of EHBF using BSP have been compared with the results from colloidal gold after correcting each of the latter for the observed gold extraction. These results show fair correlation, the figures for gold in 5 of the 10 cases being somewhat higher than the BSP results. This comparison, of course, includes only those subjects who did not have such severe hepatocellular damage that they were unable to extract BSP adequately.

TABLE IV. ESTIMATED HEPATIC BLOOD FLOW: RESULTS OBTAINED WITH BSP

PATIENT	BSP INFLOW (MG./MIN.)	PERIPHERAL BSP CONCENTRATION (MG./100 ML. PLASMA)	HEPATIC BSP EXTRACTION (%)	HEMATOCRIT (CORRECTED) (%)	EHBF (ML./MIN.)
1. C. C.	4.30	0.67	55	38	1,900
3. J. M.	4.97	0.69	77	39	1,300
8. B. W.	4.46	0.96	27	31	2,300
9. L. N.	8.26	2.80	27	30	1,600
11. R. S.	3.17	3.95	11	40	1,200
12. H. T.	3.81	2.20	13	35	2,200
22. J. G.	2.01	1.95	10	34	1,300
23. J. A.	2.52	1.75	15	48	2,300
29. G. H.	2.28	0.96	22	27	1,500
41. R. H. A.	4.61	0.86	63	38	1,300

Effect of Varying Quantities of Colloidal Gold.—In 5 subjects the extraction was determined a second time with a dose 7 to 10 times larger than the first. In two of these subjects the extraction was the same with both doses, while in the other 3 the extraction of the second (and larger) dose was more efficient (Table I).

DISCUSSION

The determination of liver blood flow from the rate of disappearance from the peripheral blood of intravenously injected colloid appeared to be a significant advance[9] since it rendered unnecessary the discomfort and difficulty of hepatic vein catheterization and was independent of the hazards of imperfect sampling. Being a simple and safe method, it could be repeated frequently in order to follow the progress of a patient. If an isotope with a gamma emitter is used,[15, 19] blood samples are not necessarily required because the disappearance constant can be determined from the external hepatic uptake, or from the decrease in radioactivity over some other area in which the colloid is not accumulating.

The studies with colloidal chromic phosphate,[14, 21] with denatured human serum albumin,[19] and the earlier work with colloidal gold[15] all gave results comparable to other methods (Table II). The more recent results with colloidal gold have given cause for concern. Krook,[17] in 1956, found the EHBF in normal subjects to be 731 ± 154 ml. per minute, while in cirrhotic patients the mean figure was 437 ml. per minute; the second series of results published by Vetter and co-workers[16] showed a mean EHBF of 770 ml. per minute for a group of 15 subjects mostly without liver disease. Riddell and associates[18] injected colloidal gold into a number of normal subjects, taking serial blood samples and counting externally over the thigh and over the liver. The disappearance constants calculated from these 3 methods were similar (0.141, 0.138, and 0.161 per minute, respectively), provided that the liver uptake curve was plotted in the usual way by subtracting serial counts over the liver from the residual constant hepatic radioactivity. Disappearance constants of this order give low values for liver blood flow, which are similar to our results and to those of Krook. However, Riddell's group found that if, in these same experiments, they took $t\frac{1}{2}$ to be the time required by the liver to accumulate half its final radioactivity, the value for k was 0.311, and for EHBF 1,582 ml. per minute. They recommended this latter procedure as the method of choice because the results were of the same order as those obtained with BSP.

We consider that this calculation is basically unsound since it depends on hepatic uptake before mixing of the colloid with the whole blood volume has taken place; the disappearance constant calculated during this period (i.e., 0.1 to 1.0 minutes after injection) is the rate of disappearance of the colloid, not from the whole blood volume, but from that restricted portion of the blood volume with which the colloid has mixed at that time.

The principles determining the application of colloid disappearance to hepatic blood flow have been discussed in detail elsewhere.[9, 13, 16, 19] In brief, if a certain colloidal preparation is to be used to measure liver flow, two conditions must be fulfilled; first, extrahepatic uptake should be negligible and, second, hepatic extraction should be 100 per cent (i.e., the liver should remove, during a single passage of blood, all the colloid presented to it). Since the spleen is in series with the liver, removal of the colloid by the spleen has the same effect as hepatic removal.

Gersh[30, 31] showed that colloidal calcium phosphate was selectively taken up by the liver and spleen in rats and dogs. Dobson and associates[32] demonstrated similar results with colloidal zirconium compounds in mice, provided that the colloid particles were large enough. Jones and co-workers,[33] in mice and dogs, and Dobson and Jones,[9] in dogs, rabbits, and mice, showed that between 80 and 98 per cent of injected colloidal chromic phosphate was localized in the liver; the last-mentioned investigators used a particle size of about 1 micron. Benacerraf and associates[13] prepared I^{131}-labeled denatured serum albumin and determined that in mice, rats, guinea pigs, and dogs, about 90 per cent of a small dose was removed from the circulation by the liver.

Colloidal gold has not been studied as extensively as have some of the other colloids. Zilversmit and associates[34] sacrificed 4 dogs about 2 hours after injection of colloidal gold (prepared by their own technique) and showed that between 84 and 89 per cent of the gold was localized in the liver and the spleen; the mean particle sizes used in these experiments were 10, 10, 20, and 27 mμ in the 4 animals, respectively. More recently it was demonstrated that in 12 dogs approximately 94 per cent of Aurocoloid (Abbott) was localized in the liver.[35]

Whereas the final localization of a colloid can be found by sacrificing an animal and measuring the radioactivity of its organs, the hepatic extraction is more difficult to determine. Dobson and Jones measured the colloidal chromic phosphate extraction directly in 2 anesthetized dogs by injecting the colloid into the portal vein and collecting all the hepatic venous blood; their results were 80 and 81 per cent.[9] The same workers injected chromic phosphate into the peripheral and the portal circulation of the rabbit and the mouse, and were able mathematically to calculate the hepatic extraction by extrapolation of the disappearance curves. They obtained values of 85 and 90 per cent for two rabbits and 79 per cent for 37 mice.

Removal of colloidal gold (Abbott radiogold) by the isolated dog liver has been studied by perfusion with fluid containing a constant concentration of gold colloid.[36] It was found that the gold extraction tended to increase with the duration of the perfusion, the mean of the lowest extractions in 9 perfusion experiments being 82 per cent.

Vetter and associates,[15] when they introduced the radiogold method for the determination of liver blood flow in human beings, measured the extraction of the colloid in 3 subjects (one with and two without impaired liver cell function) using hepatic vein catheterization. The mean values they obtained over the first 5 minutes were 87, 78, and 71 per cent, respectively. These results are in striking contrast to ours which were obtained by the same technique, using colloidal gold from the same source (Radiochemical Centre, Amersham).

The low extraction which we demonstrated in the 16 patients studied by hepatic vein catheterization provides a reason for some of the discrepancies between the calculated and the expected hepatic blood flow. If there is no extrasplanchnic removal of the colloid then the true liver blood flow equals $\frac{k \times B.V.}{E}$ where E is the extraction and B.V. the blood volume. In 3 normal subjects and in 2 cirrhotic patients the corrected EHBF (Au) corresponds well with the values obtained with Bromsulphalein (Table III). In the other cases the gold determination is far too high, a finding which can be explained only by extrasplanchnic removal of the colloid; this discrepancy appears to be most marked in patients with very severe liver disease.

Basically there are two reasons why a given colloid may not be removed completely by the liver: either the quantity of colloid employed is too large and saturates the hepatic removal mechanism, or the particle size is unsuitable.

Dobson and Jones[9] showed that variation of the dose of chromic phosphate between 0.11 and 350 μg produced no detectable alteration in the efficiency of hepatic removal, but with 500 μg the clearance was halved. Halpern and his co-workers[10-13] demonstrated that there was a critical dose of colloid below which the rate of removal was independent of the quantity injected, and above which the rate of disappearance was proportional to the administered dose. The critical dose in the instance of denatured serum albumin in man is 3.9 mg. per kilogram. It is impossible to inject into man as much as the critical dose of chromium phosphate or colloidal gold because of their instability and the toxicity of the gold.[13] The amount used by all workers estimating hepatic blood flow appears to be well below that which would saturate the liver. Actually there is evidence that in some cases prior administration of colloid increases the efficiency of hepatic removal, as in the perfusion experiments of Little and Kelly.[36] Three of the 5 subjects to whom we gave a second and larger dose of colloidal gold about 45 minutes after an earlier administration appeared to handle the second dose more efficiently. Contrary to the above experiences, Vetter and associates found that in 3 patients the rate of disappearance of 1.5 mg. colloidal gold injected 1 hour after an earlier dose of 250 μg had been cleared from the blood stream was about 20 per cent slower than before.

The relation of particle size to the rate of clearance from the blood stream and the site of localization has been studied by a number of workers. The more rapid disappearance of large particles and their selective uptake by the liver has been demonstrated for colloids of zirconium and yttrium compounds[32] and for colloidal gold[34, 37]; Zilversmit and associates[34] found a mean $t\frac{1}{2}$ of 8.2 minutes using a particle size of about 10 mμ and a $t\frac{1}{2}$ of 2.1 minutes with a particle size of about 47 mμ. Vetter's group[15] state that the gold they were using had an average particle size of 25 mμ with very little scatter around the mean, while Riddell and associates[18] reported that they had checked by electron microscopy the particle size of the gold from Amersham and that it was between 20 and 25 mμ with a small percentage of particles as small as 0.5 mμ or as large as 60 mμ. It is interesting to compare these sizes with the size of the chromic phosphate particle used by Dobson and co-workers[14] which is between 1 and 2 μ.

It appears that the best explanation for the poor hepatic extraction of colloidal gold demonstrated by us is that the particles are too small. It is difficult to reconcile our findings with those of Vetter and his colleagues without assuming that the effective particle size of the colloid from Amersham has altered since their initial work. A modification of the method of preparation could have altered either the diameter of the gold particle itself or the thickness of the surrounding gelatine coating; it is conceivable but unlikely that deterioration has occurred during air transport to Australia.

We conclude that before any colloid is used to measure liver blood flow, its extraction by the liver should be measured directly both in health and in disease, even though this involves the tedious procedure of hepatic vein catheterization. It is possible for errors due to extrasplanchnic removal to balance a reduced hepatic extraction and, thus, for plausible results to be obtained in small series; only direct extraction measurements will avoid such errors.

SUMMARY

1. Results of the measurement of hepatic blood flow with colloidal radiogold in 45 subjects are presented. They are considerably below the values found by using other well-established methods.

2. Hepatic vein catheterization in 16 subjects showed that the extraction of colloidal gold varied from patient to patient, the lowest being 8 per cent and the maximum 70 per cent. This low and inconstant extraction renders invalid the direct determination of hepatic blood flow from the rate of disappearance of this colloid from the blood stream.

3. Evidence of extrasplanchnic removal of colloidal gold was obtained in some patients with severe liver disease.

4. There is no evidence that the low extraction of colloidal gold is due to overloading of the reticuloendothelial system of the liver. In 3 subjects a second dose of colloid was extracted more efficiently than a smaller dose given 45 minutes previously.

5. It is suggested that unsuitability of the colloidal gold used in these experiments is due, principally, to an effective particle size which is too small.

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REFERENCES

1. Warren, J. V., and Brannon, E. S.: A Method of Obtaining Blood Samples Directly From the Hepatic Vein in Man, Proc. Soc. Exper. Biol. & Med. **55:** 144, 1944.
2. Bradley, S. E., Ingelfinger, F. J., Bradley, G. P., and Curry, J. J.: The Estimation of Hepatic Blood Flow in Man, J. Clin. Invest. **24:** 890, 1945.
3. Myers, J. D.: The Hepatic Blood Flow and Splanchnic Oxygen Consumption of Man. Their Estimation From Urea Production or Bromsulphalein Excretion During Catheterization of the Hepatic Veins, J. Clin. Invest. **26:** 1130, 1947.
4. Sherlock, S., Bearn, A. G., Billing, B. H., and Paterson, J. C. S.: Splanchnic Blood Flow in Man by the Bromsulphalein Method: The Relation of Peripheral Plasma Bromsulphalein Level to the Calculated Flow, J. Lab. & Clin. Med. **35:** 923, 1950.
5. Bradley, S. E., Ingelfinger, F. J., and Bradley, G. P.: Hepatic Circulation in Cirrhosis of the Liver, Circulation **5:** 419, 1952.
6. Selkurt, E. E.: Validity of the Bromsulphalein (BSP) Method for Estimating Hepatic Blood Flow, Am. J. Physiol. **175:** 461, 1953.
7. Selkurt, E. E.: Comparison of the Bromsulphalein Method With Simultaneous Direct Hepatic Blood Flow, Circulation Res. **2:** 155, 1954.
8. Edwards, A. W. T.: Sampling of Hepatic Venous Blood in the Dog, J. Appl. Physiol. **10:** 305, 1957.
9. Dobson, E. L., and Jones, H. B.: The Behavior of Intravenously Injected Particulate Material: Its Rate of Disappearance From the Blood Stream as a Measure of Liver Blood Flow, Acta med. scandinav. (supp. 273) **144:** 1-71, 1952.
10. Biozzi, G., Benacerraf, B., and Halpern, B. N.: Quantitative Study of the Granulopectic Activity of the Reticulo-endothelial System. II. A Study of the Kinetics of the Granulopectic Activity of the R.E.S. in Relation to the Dose of Carbon Injected. Relationship Between the Weight of the Organs and Their Activity, Brit. J. Exper. Path. **34:** 441, 1953.
11. Benacerraf, B., Stiffel, C., Biozzi, G., and Halpern, B. N.: Mesure de l'activité granulopexique du système réticulo-endothélial à l'aide due saccharate d'oxyde de fer, chez le rat, Compt. rend. Soc. de biol. **148:** 186, 1954.
12. Halpern, B. N., Benacerraf, B., Biozzi, G., and Stiffel, C.: Facteurs régissant la fonction phagocytaire due système réticulo-endothélial, Rev. hémat. **9:** 621, 1954.

13. Benacerraf, B., Biozzi, G., Halpern, B. N., Stiffel, C., and Mouton, D.: Phagocytosis of Heat-denatured Human Serum Albumin Labeled With I^{131} and Its Use as a Means of Investigating Liver Blood Flow, Brit. J. Exper. Path. **38:** 35, 1956.
14. Dobson, E. L., Warner, C. F., Finney, C. R., and Johnston, M. E.: The Measurement of Liver Circulation by Means of the Colloid Disappearance Rate, Circulation **7:** 690, 1953.
15. Vetter, H., Falkner, R., and Neumayr, A.: The Disappearance Rate of Colloidal Radiogold From the Circulation and Its Application to the Estimation of Liver Blood Flow in Normal and Cirrhotic Subjects, J. Clin. Invest. **33:** 1594, 1954.
16. Vetter, H., Grabner, G., Höffer, R., Neumayr, A., and Parzer, O.: Comparison of Liver Blood Flow Values Estimated by the Bromsulphalein and by the Radio-gold Method, J. Clin. Invest. **35:** 825, 1956.
17. Krook, H.: Circulatory Studies in Liver Cirrhosis, Acta med. scandinav. Suppl. 318, 1956.
18. Riddell, A. G., Griffiths, D. B., McAlister, J. M., and Osborn, S. B.: The Measurement of Liver Blood Flow With Colloidal Radiogold (198Au), Clin. Sc. **16:** 315, 1957.
19. Biozzi, G., Benacerraf, B., Halpern, B. N., Stiffel, C., and Hillemand, B.: Exploration of the Phagocytic Function of the Reticuloendothelial System With Heat Denatured Human Serum Albumin Labelled With I^{131} and Application to the Measurement of Liver Blood Flow in Normal Man and in Some Pathological Conditions, J. Lab. & Clin. Med. **51:** 230, 1958.
20. Dobson, E. L., Warner, G. F., Finney, C. R., and Adamson, D. M.: Extrasplanchnic Blood Volume: Its Importance in Calculation of Liver Blood Flow From Colloid Disappearance Rate, Federation Proc. (Abstract 160) **15:** 51, 1956.
21. Nardi, G. L.: Effect of Splenorenal Shunts on Estimated Hepatic Blood Flow, A. M. A. Arch. Surg. **70:** 530, 1955.
22. Falkner, R., Neumayr, A., and Vetter, H.: Die Bestimmung des Blutflusses durch die Leber mit kolloidalem Radiogold. Symposium on Clinical Applications of Radioactive Isotopes, Bad Gastein, January, 1954, Strahlenther.
23. Gaebler, O. H.: Determination of Bromsulphalein in Normal, Turbid, Hemolysed or Icteric Serums, Am. J. Clin. Path. **15:** 452, 1945.
24. Wilkins, R. W., Culbertson, J. W., and Rymut, A. A.: The Hepatic Blood Flow in Resting Hypertensive Patients Before and After Splanchnicectomy, J. Clin. Invest. **31:** 529, 1952.
25. Bradley, S. E., Smythe, C. M., Fitzpatrick, H. F., and Blakemore, A. H.: The Effect of a Portacaval Shunt on Estimated Hepatic Blood Flow and Oxygen Uptake in Cirrhosis, J. Clin. Invest. **32:** 526, 1953.
26. Shackman, R., Craber, I. G., and Melrose, D. G.: Liver Blood Flow and General Anaesthesia, Clin. Sc. **12:** 307, 1953.
27. Edwards, A. W. T.: Studies in Portal Hypertension, Estimated Portal Pressure and Hepatic Blood Flow in Patients With Cirrhosis of the Liver and Other Disorders, M. J. Australia **1:** 671, 1955.
28. Redeker, A. G., Geller, H. M., and Reynolds, T. B.: Hepatic Wedge Pressure, Blood Flow, Vascular Resistance and Oxygen Consumption Before and After End-to-Side Portacaval Shunt, J. Clin. Invest. **37:** 606, 1958.
29. Fauvert, A., Benhamau, J.-P., Nicollo, S., and Loverdo, A.: La clearance de l'or colloidal radio-actif (198Au). I. Valeurs Normales et valeurs pathologiques, Rev. franç. étud. clin. et biol. **3:** 762, 1958.
30. Gersh, I.: Histochemical Studies of the Fate of Colloidal Calcium Phosphate in the Rat, Anat. Rec. **70:** 331, 1938.
31. Gersh, I.: Fate of Colloidal Calcium Phosphate in the Dog, Am. J. Physiol. **121:** 589, 1938.
32. Dobson, E. L., Gofman, J. W., Jones, H. B., Kelly, L. S., and Walker, L. A.: Studies With Colloids Containing Radioisotopes of Yttrium, Zirconium, Columbium, and Lanthanum, J. Lab. & Clin. Med. **34:** 305, 1949.
33. Jones, H. B., Wrobel, C. J., and Lyons, W. R.: A Method of Distributing Beta-Radiation to the Reticulo-endothelial System and Related Tissues, J. Clin. Invest. **23:** 783, 1944.
34. Zilversmit, D. B., Boyd, G. A., and Brucer, M.: The Preparation and Particle Size Measurement of Monodisperse Radioactive Gold Colloids, J. Lab. & Clin. Med. **40:** 261, 1952.
35. Ganz, A., and Brucer, M.: Blood Clearance and Tissue Distribution Studies in the Dog Following Intravenous Administration of Radioactive Colloidal Gold (Aurcoloid), J. Lab. & Clin. Med. **52:** 20, 1958.
36. Little, R. C., and Kelly, H. B.: Removal of Radioactive Gold Colloid by the Perfused Mammalian Liver, Am. J. Physiol. **173:** 265, 1953.
37. Sheppard, C. W., Jordan, G., and Hahn, P. F.: Disappearance of Isotopically Labelled Gold Colloids From the Circulation of the Dog, Am. J. Physiol. **164:** 345, 1951.

PROTEIN, HEXOSE, AND HEXOSAMINE IN THE NONDIALYZABLE FRACTION OF THE FILTERED URINE IN NORMAL YOUNG MEN

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RECENT studies have provided clinical and experimental knowledge on protein-bound carbohydrate from cartilage,[40] connective tissue,[29, 31] bone,[12, 13] serum,[7, 14, 17, 28, 45] and urine.[3, 4, 8, 9, 15, 19, 22, 42] While much work has been done on urine, there still is no standard procedure available for the fractionation of its several protein and carbohydrate components.

The aim of this paper is to introduce a simple method for quantitative determination of some components of the nondialyzable fraction of filtered urine of normal men.

MATERIALS AND METHODS

Fifteen normal men, 22 to 29 years old, were studied. Twenty-four hour urine specimens were collected, stored at 0° C. without the addition of preservatives, and assayed as soon as possible after the end of the collection period. Completeness of collection was checked through the 24-hour endogenous creatinine excretion.[20] The urine was filtered on Schleicher-Schuell No. 595 filter paper and an aliquot was dialyzed for 24 hours in cellophane tubing ("dialyzer membranes," wall thickness 0.00072 inch) against tap water at 0° C. The contents of the dialysis bag were brought to a known volume with distilled water and used for chemical determinations.

A scheme of the chemical fractionation procedure and the nomenclature of the fractions so obtained are given in Table I.

Analytic Methods.—

Hexose: The anthrone method of Seifter and associates[39] was modified by the addition of 5 per cent boric acid to stabilize the anthrone reagent.[25] This method also detects hexuronic acid. Standards containing equimolecular amounts of galactose and mannose were run simultaneously with the unknowns. Readings were performed at 620 mμ.

Hexosamine: Hexosamine was determined according to either the method of Shetlar and co-workers[41] or that of Rondle and Morgan[37]; the results of both techniques agreed satisfactorily. Standards of glucosamine were used, and readings were made at 540 mμ.

Protein: Protein was estimated by the method of Lowry and associates,[24] using human serum albumin* for standardization readings being taken at 700 mμ. Spectrophotometry was performed in a Coleman Junior apparatus.

Experimental.—The following experiments were performed on normal urine and are reported together with a few comments on the different steps of the procedure.

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TABLE I. NONDIALYZABLE FRACTION OF THE FILTERED URINE: CHEMICAL FRACTIONATION AND NOMENCLATURE

NAME	CONSTITUENTS ASSAYED	AMOUNT USED* (RANGE)	CHEMICAL SEPARATION TECHNIQUE
Total carbohydrate	Hexose Hexosamine	0.2-1.0 ml. of the prepared urine	None (direct determination)
Carbohydrate bound to protein	Hexose Hexosamine	4.0-10.0 ml. of the prepared urine	1 vol. of 5% phosphotungstic acid in 2 N HCl + 1 vol. of the prepared urine. The precipitate is assayed
Total protein	Protein	2.0-3.0 ml. of the prepared urine	
Carbohydrate bound to protein soluble in perchloric acid	Hexose Hexosamine	10.0-15.0 ml. of perchloric filtrate	2 vol. of 6% perchloric acid + 1 vol. of the prepared urine. Filtration with Whatman No. 50 paper. 1 vol. of filtrate + 1 vol. of 5% phosphotungstic acid. The precipitate is used
Protein soluble in perchloric acid	Protein	3.0-6.0 ml. of the perchloric filtrate	
Carbohydrate not bound to protein	Calculation	By difference between total carbohydrate and carbohydrate bound to protein	
Carbohydrate bound to protein not soluble in perchloric acid	Calculation	By difference between carbohydrate bound to protein and carbohydrate bound to protein soluble in perchloric acid	
Protein not soluble in perchloric acid	Calculation	By difference between total protein and protein soluble in perchloric acid	

*Original urine diluted to the proportion of 80/100 ml.

Filtration: When the urine was filtered, a component containing hexose and protein was retained. This component accounted for 15 to 20 per cent of the total urinary protein (3 normal specimens studied) and had the following characteristics: it sedimented at 4,000 r.p.m., was soluble in 6 per cent perchloric acid and in 15 per cent trichloroacetic acid, did not coagulate on heating to the boiling point after solution in distilled water, was precipitated by 5 per cent phosphotungstic acid in 2 N HCl, and contained protein and hexose in a ratio of 6.8 to 7.8 and no detectable inorganic phosphate.

Dialysis: This was found to be necessary because there are substances in the urine which interfere with the determinations of hexose and hexosamine by the methods used. After dialysis the urine was kept in a freezer at -20° C. Freezing did not interfere with the results of the fractionation if the samples were thawed only once. No significant differences in the results were found when 9 urines were analyzed immediately after collection or after storage for up to 3 months at -20° C.

Carbohydrate not bound to protein: The dialyzed filtered urine was precipitated with 5 per cent phosphotungstic acid in 2 N HCl and filtered with Whatman No. 50 filter paper or centrifuged. The supernatant was precipitated with ethyl alcohol 1:4 (vol./vol.). The resultant precipitate, a white powder when dried, was extremely soluble in water, giving positive reactions for hexose and hexosamine, was negative for protein, and showed metachromasia with toluidine blue solution. The values obtained by difference were used for they were less subject to error.

Carbohydrate bound to protein not soluble in 6 per cent perchloric acid: This fraction was determined in 12 cases in order to check the values obtained by difference. They were in close agreement. The mathematical derivation was used because of its simplicity.

Reproducibility of the results after dialysis: On triplicate dialyses of the same urine an average value (milligrams per 24-hour volume) ± standard deviation was found of 126.0 ± 5.0 for total protein, 158.7 ± 7.4 for total hexose, and 20.6 ± 1.9 for hexose bound to protein. The range of results is within satisfactory variation (10 per cent).

TABLE II. CARBOHYDRATE CONTENT AS MEASURED BY ITS HEXOSE, HEXOSAMINE, AND PROTEIN FROM THE PREPARED URINE OF NORMAL MEN (22 TO 29 YEARS OLD) EXPRESSED AS MG. PER 24-HOUR VOLUME

	HEXOSE (15 CASES)	HEXOSAMINE (14 CASES)	PROTEIN (12 CASES)
Total carbohydrate	275.4 ± 51.98* (178.75-368.5)†	67.7 ± 9.31 (51.3-83.2)	
Carbohydrate not bound to protein	249.5 ± 49.7 (156.4-333.1)	46.7 ± 8.49 (22.5-57.4)	
Carbohydrate bound to protein	25.8 ± 4.3 (20.3-34.4)	20.6 ± 5.69 (12.0-32.0)	
Total protein			126.3 ± 21.7 (90.0-167.8)
Carbohydrate bound to protein soluble in perchloric acid	20.2 ± 2.9 (17.5-26.3)	12.1 ± 3.11 (6.6-17.6)	
Protein soluble in perchloric acid			86.4 ± 19.2 (63.0-130.4)
Carbohydrate bound to protein not soluble in perchloric acid	5.6 ± 2.5 (2.3-9.7)	8.6 ± 3.99 (5.4-17.9)	
Protein not soluble in perchloric acid			39.9 ± 13.1 (27.0-76.8)

*Standard deviation.
†Range.

Reproducibility of the determination of protein and hexose: Six determinations gave an average value (milligrams per 24-hour volume) ± standard deviation of 158.7 ± 8.4 for total protein, 224.2 ± 6.04 for total hexose, and 23.4 ± 0.74 for hexose bound to protein. The greatest variation obtained was 10 per cent.

RESULTS

The average, standard deviation, and range of normal urine fractions are found in Table II.

As it can be seen, the major carbohydrate component of the dialyzed filtered urine was not bound to protein and ranged from 87.4 to 93.3 per cent

TABLE III. HEXOSE-HEXOSAMINE, PROTEIN-HEXOSE + HEXOSAMINE AND PROTEIN-HEXOSE RATIOS IN THE PREPARED URINE FRACTIONS (10 SUBJECTS)

	HEXOSE-HEXOSAMINE	PROTEIN-HEXOSE + HEXOSAMINE	PROTEIN-HEXOSE
Carbohydrate not bound to protein	5.430* (3.629-6.372)†		
Carbohydrate bound to protein soluble in perchloric acid	1.757 (1.440-2.803)	2.638 (2.055-3.498)	
Protein soluble in perchloric acid			4.160 (3.384-4.958)
Carbohydrate bound to protein not soluble in perchloric acid	0.7495 (0.44-1.123)	3.038 (1.678-5.130)	
Protein not soluble in perchloric acid			7.399 (4.110-13.479)

*Standard deviation.
†Range.

of the total carbohydrate (as hexose); the major protein component is the one soluble in 6 per cent perchloric acid corresponding to 68.9 ± 7.8 per cent of the total protein.

The study of the hexose-hexosamine ratio (Table III) disclosed significant differences ($P=0.05$)[14] between the 3 fractions analyzed. The ratios were 0.4 to 1.1, 1.4 to 2.8, and 3.6 to 6.4, respectively, for carbohydrate bound to protein insoluble in perchloric acid, carbohydrate bound to protein soluble in perchloric acid, and carbohydrate not bound to protein. On the other hand when the ratios of protein-hexose + hexosamine and protein-hexose were compared (Table III), no significant differences were found between the fractions soluble and insoluble in 6 per cent perchloric acid, but the first one gave less variable ratios.

DISCUSSION

The present study pertains only to filtered urine subject to thorough dialysis, and excludes 2 other carbohydrate-containing components: (1) the one which is retained on the filter paper, and (2) the low molecular weight carbohydrate which probably is dialyzed out, as previously reported by Boas.[6]

In the dialyzed filtered urine 3 components were studied.

1. Carbohydrate Not Bound to Protein.—This is the major carbohydrate component of urine and has the following characteristics: (1) it does not precipitate with 5 per cent phosphotungstic acid in 2 N HCl, (2) its hexose-hexosamine ratio is 3.6 to 6.4, (3) it precipitates with absolute ethanol, and (4) it gives a positive test with toluidine blue.[1]

According to Meyer[30, 32] this component should be an acid mucopolysaccharide but it may also contain a neutral polysaccharide. Rigas and Heller,[36] using free electrophoresis, were the first to report the presence of a carbohydrate not bound to protein in dialyzed urine. This component had no mobility at pH 8.6, gave a positive reaction for carbohydrate, and was negative for protein. By paper electrophoresis at pH 4.5, Markham and associates[25] found 2 fast-moving fractions, both of which were positive for carbohydrate, in addition to a slower-moving one containing tyrosine. In the dialyzed urine of normal men, Hamerman and Hatch[18] found that a fraction existed which contained hexose and hexosamine but which did not precipitate with phosphotungstic acid. A brown powder which was called "mucoprotein B" by Anderson,[2] obtained after benzoic acid adsorption and ethanol precipitation, gave positive tests for carbohydrate and negative for protein. The same component was studied by Kerby[21] and DiFerranti[11] through its hexuronic acid content.

2. Carbohydrate Bound to Protein Soluble in Perchloric Acid.—As can be seen from the classification of Meyer[30, 32] and Morgan,[33] this component cannot be called a glycoprotein for it contains more than 4 per cent hexosamine; we do not know whether it belongs to the mucoprotein or mucoid groups. Beckman and associates[5] in normal urine and Popenoe[35] in cases of nephrotic syndrome found a component with the same chemical characteristics of this

fraction. The component studied by Popenoe behaved immunochemically and electrophoretically as mucoprotein-1 (Mp-1); its molecular weight was 44,000. The author asserts that this component is different from Tamm and Horsfall's[43] mucoprotein. Working with serum and urine, Markham and associates,[25] by paper electrophoresis at pH 4.5, found two fractions (M_1 and M_2) with a lower isoelectric point than albumin which corresponded to alpha-1 and alpha-2 globulin fractions studied at pH 8.6. This work confirmed the findings of Laurell[23] of a great heterogeneity of these fractions.

3. Carbohydrate Bound to Protein Insoluble in 6 Per Cent Perchloric Acid.—We could not find, in the literature, any previous reference to this component. Rigas and Heller[36] and McGarry and associates[27] suggest that albumin, and beta and gamma globulins may belong to this fraction. It is doubtful whether the alpha globulin, from the perchloric acid soluble fraction, contributes to this component. In accordance with King and associates[22] the protein moiety constitutes 47 per cent of the total nondialyzable solids in the human urine. The total protein excretion in our cases, including that fraction retained on filter paper, was only slightly lower than that obtained by King.

The nomenclature and definition of the above-reviewed components cannot be established until more is known about their chemical characterization, biologic identification, and origin. As to the latter there are three hypotheses:

A. Urinary tract origin: Boyce and Garvey[10] believe that the "mucopolysaccharide of urinary calculi may derive from connective tissue and epithelia of the urinary tract. The mucoprotein would arise from transitional epithelium but would be different from the normal secretory product of these cells."

B. Serum origin: McGarry and associates[27] believe that the urinary albumin originates from serum. The fact that alpha-1 acid glycoprotein from urine is similar to that of serum has been confirmed by Popenoe[35] and Rigas and Heller[36] who obtained electrophoretically almost the same pattern (albumin, alpha, beta, and gamma globulins) from normal serum and urine.

C. Serum origin after metabolization by tubular epithelium: Sellers[38] believes that serum proteins are to some extent filtered, reabsorbed, and catabolized by tubular cells. Through this mechanism, polypeptides and amino acids would be retained. It cannot be excluded that after filtration through the glomeruli, the glycoprotein would be dissociated into carbohydrate and protein by the activity of the tubules. The first moiety would be excreted and the second one reabsorbed. The positivity to the histochemical PAS reaction of the droplets found in the renal cells of human beings and experimental animals has been stressed by Oliver and co-workers.[34] These authors also suggest that this PAS reaction could be due to filtered and reabsorbed glycoproteins from blood plasma.

Physiologic and pathologic conditions are being studied in this laboratory aimed at achieving a better characterization of these fractions.

SUMMARY

A simple method for fractionation of dialyzed filtered urine is presented; two fractions were not studied, namely, the one retained on the filter paper and the one which is supposed to be dialyzed out.

Three main fractions were obtained from the nondialyzable material:

1. A carbohydrate not bound to protein which is tentatively identified as a mucopolysaccharide.

2. A carbohydrate bound to protein, soluble in 6 per cent perchloric acid, and precipitated by 5 per cent phosphotungstic acid in 2 N hydrochloric acid. This component is probably a mucoid or a mucoprotein.

3. Protein and carbohydrate bound to protein, insoluble in 6 per cent perchloric acid. This fraction would correspond to albumin and beta and gamma globulins.

The 3 fractions have been determined in the urine of 15 normal men, 22 to 29 years old.

REFERENCES

1. Albeaux-Fernet, M., Bugard, P., and Romani, J. D.: Étude de la mucoprotéinurie dans les collagénoses, les endocrinopathies et les traitements hormonaux, Presse méd. **63**: 509-511, 1955.
2. Anderson, A. J.: A Method for the Isolation of Urinary Mucoproteins, Biochem. J. **56**: Proc. 25, 1954.
3. Anderson, A. J., Lockey, E., and MacLagan, N. F.: Some Biological Properties of Urinary Mucoproteins, Biochem. J. **60**: Proc. 40, 1955.
4. Anderson, A. J., and MacLagan, N. F.: The Isolation and Estimation of Urinary Mucoproteins, Biochem. J. **59**: 638, 1955.
5. Beckman, W. W., Hiller, A., Shedlowsky, T., and Archibald, R. M.: The Occurrence in Urine of a Protein Soluble in Trichloroacetic Acid, J. Biol. Chem. **148**: 147-148, 1943.
6. Boas, N. F.: Low Molecular Weight Hexosamine-Containing Compounds in Urine, Proc. Soc. Exper. Biol. & Med. **92**: 122-124, 1956.
7. Bollet, A. J.: Acid Mucopolysaccharides in Normal Serum, J. Clin. Invest. **36**: 1328-1332, 1957.
8. Boyce, W. H., Garvey, F. K., and Norfleet, C. M., Jr.: Proteins and Others Biocolloids of Urine in Health and in Calculous Disease. I. Electrophoretic Studies at pH 4.5 and 8.6 of Those Components Soluble in Molar Sodium Chloride, J. Clin. Invest. **33**: 1287-1297, 1954.
9. Boyce, W. H., and Swanson, M.: Biocolloids of Urine in Health and in Calculous Disease. II. Electrophoretic and Biochemical Studies of a Mucoprotein Insoluble in Molar Sodium Chloride, J. Clin. Invest. **34**: 1581-1589, 1955.
10. Boyce, W. H., and Garvey, F. K.: The Amount and Nature of the Organic Matrix in Urinary Calculi (a Review), J. Urol. **76**: 213-227, 1956.
11. DiFerranti, N., and Rich, C.: The Determination of Acid Aminopolysaccharides in Urine, J. Lab. & Clin. Med. **48**: 491-494, 1956.
12. Duthie, R. B., and Baker, A. N.: An Autoradiographic Study of Mucopolysaccharides and Phosphate Complexes in Bone Growth and Repair, J. Bone & Joint Surg. **373**: 304-323, 1955.
13. Glegg, R. E.: A Method for Fractionating the Carbohydrate Components of Bone, Arch. Biochem. **55**: 19-24, 1955.
14. Goa, J.: On Protein-bound Carbohydrate in Human Serum, Scandinav. J. Clin. & Lab. Invest. **7** (Suppl. 22): 1955.
15. Gottschalk, A.: Carbohydrate Residue of a Urine Mucoprotein Inhibiting Influenza Virus Haemagglutination, Nature **170**: 662, 1952.
16. Goulden, C. H.: Methods of Statistical Analysis, ed. 2, New York, 1952, John Wiley & Sons, Inc.
17. Greenspan, E. M.: Clinical Significance of Serum Mucoprotein, Advances Int. Med. **7**: 101-123, 1955.
18. Hamerman, D., and Hatch, F. T.: Urinary Levels of Mucoprotein in Normal Male Subjects, Proc. Soc. Exper. Biol. & Med. **89**: 279-280, 1955.

19. Hamerman, D., Hatch, F. T., Reife, A., and Bartz, K. W.: Non-dialyzable Material in Normal Human Urine, J. LAB. & CLIN. MED. **46**: 848-856, 1955.
20. Haugen, H. N., and Blegen, E. M.: The True Endogenous Creatinine Clearance, Scandinav. J. Clin. & Lab. Invest. **5**: 67-71, 1953.
21. Kerby, G. P.: The Excretion of Glucuronic Acid and Acid Mucopolysaccharides in Normal Human Urine, J. Clin. Invest. **33**: 1168-1174, 1954.
22. King, J. S., Jr., Boyce, W. H., Little, J. M., and Artom, C.: Total Non-dialyzable Solids (TNDS) in Human Urine. I. The Amount and Composition of TNDS From Normal Subjects, J. Clin. Invest. **37**: 315-321, 1958.
23. Laurell, H.: Demonstration of Two New Electrophoretic Components in Serum From a Patient With Rheumatoid Arthritis, Ark. Kemi. **3**: 115-116, 1951.
24. Lowry, O. H., Rosenbourgh, N. J., Farr, A. L., and Randall, R. J.: Protein Measurement With the Folin Phenol Reagent, J. Biol. Chem. **193**: 265-275, 1951.
25. Markham, R. L., Jacobs, J. H., and Fletcher, E. T. D.: Zone Electrophoresis of Serum and Urine at pH 4.5 and Its Application to the Isolation and Investigation of Mucoproteins, J. LAB. & CLIN. MED. **48**: 559-570, 1956.
26. Martirani, I., Hoxter, G., Wajchenberg, B. L., Mariani, I., and Cintra, A. B. U.: Dosagem das glicoproteinas totais (não glucosamínicas) do sôro e da urine com o reagente de antrona modificado, Rev. paulista med. **47**: 576, 1955.
27. McGarry, E., Sehon, A. H., and Rose, B.: The Isolation and Electrophoretic Characterization of the Protein in the Urine of Normal Subjects, J. Clin. Invest. **34**: 832-844, 1955.
28. Mehl, J. W., Golden, F., and Winzler, R. J.: Mucoproteins of Human Plasma. IV. Electrophoretic Demonstration of Mucoproteins in Serum pH 4.5, Proc. Soc. Exper. Biol. & Med. **72**: 110-114, 1949.
29. Meyer, K.: Chemistry of Connective Tissue: Polysaccharides. The Connective Tissue. Transactions of the First Conference, New York, 1950, Josiah Macy, Jr., Foundation.
30. Meyer, K.: In Cole, W. H., editor: Mucoproteins and Mucoids in Some Conjugated Proteins, New Brunswick, 1953, Rutger's University Press.
31. Meyer, K.: The Mucopolysaccharides of the Ground Substance of Connective Tissue, Science **113**: 596, 1951.
32. Meyer, K.: Problems of Classification, in George F. Springer, editor: Polysaccharides in Biology. Transactions of the First Conference, New York, 1956, Josiah Macy, Jr., Foundation.
33. Morgan, W. T. J.: Some Aspects of the Chemistry of Mucins, Proc. Roy. Soc. Med. **46**: 783-785, 1953.
34. Oliver, J., Straus, W., Kretchmer, N., Lee, Y. C., and Cherot, F.: The Histochemical Characteristics of Absorption Droplets on the Nefron, J. Histochem. **3**: 277-283, 1955.
35. Popenoe, E. A.: Characterization of a Glycoprotein in the Urine of Patients With Proteinuria, J. Biol. Chem. **217**: 61-66, 1956.
36. Rigas, D. A., and Heller, C. G.: The Amount and Nature of Urinary Proteins in Normal Human Subjects, J. Clin. Invest. **30**: 853-861, 1951.
37. Rondle, C. J. M., and Morgan, W. T. J.: The Determination of Glucosamine and Galactosamine, Biochem. J. **61**: 586, 1955.
38. Sellers, A. L.: The Mechanism and Significance of Protein Excretion by Normal Kidney, A. M. A. Arch. Int. Med. **98**: 801-806, 1956.
39. Seifter, S., Seymour, D., Novic, B., and Muntwyler, E.: The Estimation of Glycogen With the Anthrone Reagent, Arch. Biochem. **25**: 191-200, 1950.
40. Shatton, J., and Maxwell, S.: Isolation of a Mucoprotein From Cartilage, J. Biol. Chem. **211**: 565, 1954.
41. Shetlar, M. R., Foster, J. V., Kely, K. H., and Everett, M. R.: Serum Polysaccharide Level in the Normal State, Proc. Soc. Exper. Biol. & Med. **69**: 507-511, 1948.
42. King, J. S., Jr., and Hyder, N.: A Glucosamine Congugate Occurring in Human Urine, Proc. Soc. Exper. Biol. & Med. **89**: 342-345, 1955.
43. Tamm, I., and Horsfall, F. L., Jr.: A Mucoprotein Derived From Human Urine Which Reacts With Influenza, Mumps and New Castle Disease Virus, J. Exper. Med. **95**: 71-97, 1952.
44. Winzler, R. J., Devor, A. W., Mehl, J. W., and Smyth, I. M.: Studies on the Mucoproteins of Human Plasma. I. Determination and Isolation, J. Clin. Invest. **27**: 609, 1948.
45. Winzler, R. J.: In Glick, David, editor: Determination of Serum Glycoprotein in Methods of Biochemical Analysis, New York, 1955, Interscience Publishers, Inc., vol. II.

A NEW PARALLEL TUBE CONTINUOUS HEMODIALYZER

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FOLLOWING the first clinically successful hemodialysis unit constructed by Kolff and Berk,[1] a number of units of varying designs have been reported.[2-8] All of these presently available artificial kidneys can be divided into two groups both of which are sufficiently efficient in terms of dialyzing capabilities. One group exemplified by the rotating drum unit (Kolff-Brigham) acts only as a dialyzer because of the low hydrostatic pressure within the cellulose tubing. This unit has the major disadvantages of requiring assembling and sterilization immediately prior to use and a relatively labile extracorporeal blood volume. Furthermore, any reduction of extracellular fluid volume, when desired, can only be achieved by the use of hyperosmolar dialyzing solutions. In the second variety, exemplified by the Kolff twin coil kidney, the Skeggs-Leonard unit, the Alwall unit, and others, the dialyzing membrane is supported and compressed within screens, grids, corrugated pads, or trays. This external compression necessitates a considerable increase in the hydrostatic pressure to propel the blood through the unit with a proportionate increase in damage to the cellular elements of the blood, possible rupture of the membrane, and a considerable loss of plasma water via ultrafiltration which may not be warranted and which must be replaced. These supports or containers also restrict the continuous clear observation of all the extracorporeal blood channels.

It has been our aim, therefore, to develop a safe, relatively transparent, disposable hemodialyzing unit which will operate efficiently either with or without significant ultrafiltration. The unit must also contain a reasonably limited extracorporeal blood volume which changes relatively little over a wide range of in vivo conditions. In addition to these characteristics, the ideal clinical unit should incorporate the following desirable features: (1) easy sterilization, (2) unit can be tested for leaks prior to priming with blood, (3) rapid exclusion and replacement of defective membrane without interruption of dialysis, (4) surface area adaptable to needs, (5) reasonable compactness and ease in assembly, (6) relatively inexpensive, (7) "long term" shelf life.

This report describes our experimental results with a newly designed hemodialyzer which presently incorporates, to a greater or lesser degree, most of the above features. The unit consists of parallel lengths of Visking cellulose tubing which are internally supported by inert plastic rods, the total

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number depending on the desired membrane surface area. In the following experiments, the entire unit consisted of 16 such components, with a membrane surface area of 1.0 M.².

METHODS

Details of Design.—In order to avoid the problems associated with external supports, and yet not decrease the surface area to volume ratio, space-filling rods of an inert material were inserted into cellulose tubing (Visking 27/32 inch, outside diameter 0.001 inch thickness). These inserts were made from a 36 inch extruded methyl methacrylate (Lucite, Plexiglas) rod with an original outside diameter of ⅞ inch. These were centerless ground to a relatively uniform outside diameter of 0.826 ± 0.002 inch (Fig. 1).

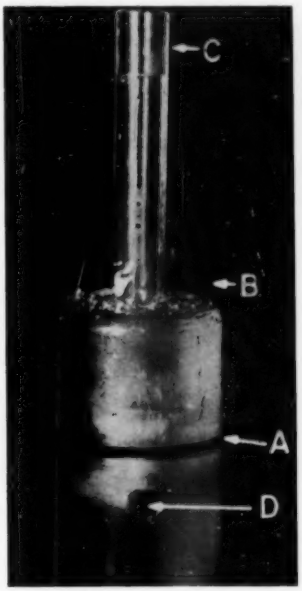

Fig. 1.—Rod threaded with cellulose tubing. *A* = 0 silk ligature seal. *B* = Cellulose tubing. *C* = Nozzle with inside Luer taper. *D* = Blood channel.

Appropriate lengths of cellulose tubing were washed free of plasticizer and then inflated with air to 160 to 180 mm. Hg in order to detect leaks. With the aid of some air pressure to distend the tubing, the satisfactory lengths of tubing were threaded over each rod. The ends were sealed with 6 winds of zero silk (Fig. 1). Each component was again tested for leaks by inflation to 150 mm. Hg. The residual space between the cellulose tubing and the rod was calculated to be about 0.5 mm. (vide infra).

The 16 components were mounted horizontally in a large transparent tank (Fig. 2, *A*) with inside measurements of 47 by 14 by 9 inches and a capacity of 90 liters. The ends of each component were appropriately connected to the arterial (Fig. 2, *B*) or venous manifold (Fig. 2, *C*) or to each other, by a standard male Luer nylon fitting* attached to nontoxic polyvinyl chloride tubing. The arrangement of the 16 components in 8 parallel channels permitted the removal and replacement of any component from the unit after clamping off the plastic tubing at each end.

*The male Luer nylon fittings were generously supplied by the Abbott Laboratories, Chicago, Ill.

The mounting racks (Fig. 2, *D*) were freely movable in a vertical track (Fig. 2, *E*). One of the racks was suspended from a cam (Fig. 2, *F*) with a 1 inch vertical displacement. This cam was activated by a variable speed 1/18 horsepower motor at 100 to 120 r.p.m.

Dialyzing solution, 220 to 260 L., was recirculated at 30 L. per minute by a centrifugal pump between a standard 55 gallon stainless steel drum and the large tank. In order to maintain the bath solution at 38° C., the drum was fitted with a thermostatically controlled heater. Whereas tap water served as the dialyzing solution for the in vitro studies, a balanced electrolyte solution was used for the dog experiments. This solution contained sodium 135 mEq./L., potassium 3 mEq./L., bicarbonate 35 mEq./L., chloride 105 mEq./L., and calcium 3 to 5 mEq./L. A Beckman Zeromatic pH meter with appropriate electrodes continuously monitored the pH of the solution which was maintained at 7.35 to 7.55 by adding sufficient lactic acid and gaseous CO_2.

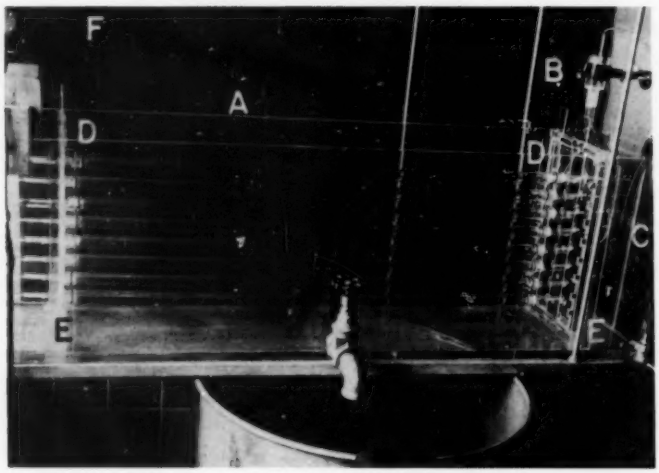

Fig. 2.—Assembled unit with 1.0 M.² surface area. *A* = Tank; inside measurements 47 by 14 by 9 inches, capacity 90 L. *B* = Arterial manifold. *C* = Venous manifold. *D* = Mounting rack. *E* = Vertical track. *F* = Eccentric cam with suspension.

Chemical Methods.—The urea concentration was determined by a modification of the urease method of Van Slyke-Cullen.[9] The concentration of creatinine in aqueous solution as used in the in vitro studies was determined directly, or if necessary after appropriate dilution, by the alkaline picrate colorimetric technique as outlined by Brod and Sirota.[10]

RESULTS

In Vitro.—Eight satisfactory in vitro experiments were performed utilizing a well-mixed 10 or 20 L. of an aqueous urea or urea-creatinine solution with an initial concentration of 240 to 260 mg. per cent urea nitrogen and 11 to 13 mg. per cent creatinine. This solution was recirculated through the unit. Mean inflow tract pressures were measured continuously by either a damped mercury or a calibrated aneroid manometer. Periodically, a small aliquot was withdrawn from the inflow tract for chemical analysis. Shortly thereafter, the delay depending upon the rate of flow through the apparatus, a timed collection of the entire outflow was measured in a volumetric cylinder. A small portion of this efflux was utilized for chemical analysis while the rest was returned to the source.

Twenty-four determinations of the extraction fraction for urea nitrogen and creatinine at various flow rates were performed in which the unit was not agitated by the motor cam suspension. Thirty-two determinations were similarly

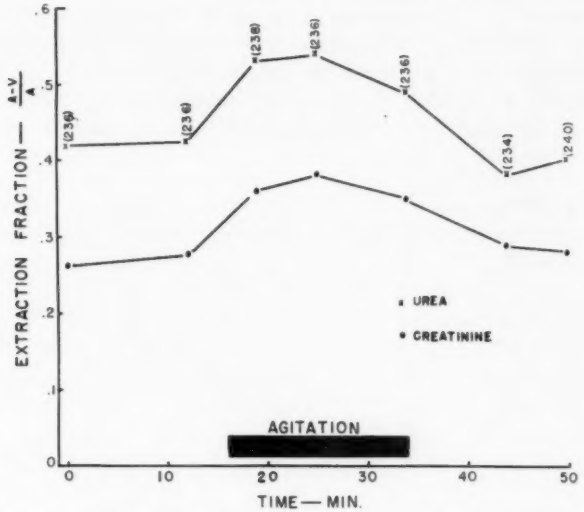

Fig. 3.—The results in one experiment demonstrating the improvement in the extraction fraction for urea and creatinine with agitation. Flow through the unit was kept relatively constant as indicated by the numbers within the parentheses.

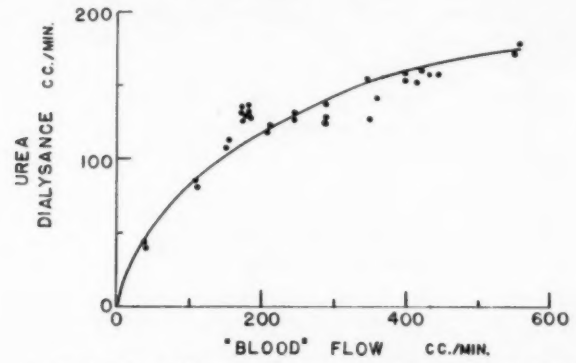

Fig. 4.—The relationship between urea dialysance and blood flow.

made in which agitation was utilized. In the former series of observations, these values ranged from 0.77 to 0.22 for urea nitrogen and 0.42 to 0.17 for creatinine at flow rates of 44 to 376 ml. per minute. The tank concentrations ranged from 0 to 10 mg. per cent urea nitrogen and 0 to 0.6 mg. per cent creatinine. With agitation, the extraction fraction varied from 0.92 to 0.30 for urea nitrogen and 0.80 to 0.24 for creatinine at flow rates of 44 to 420 ml. per minute. In this series tank concentrations for urea nitrogen and creatinine ranged from 0 to

15 mg. per cent and 0 to 0.70 mg. per cent, respectively. The results in one experiment showing the increased extraction with agitation while the flow rate remained constant are plotted in Fig. 3. The cause for this variability was thought to be due, when agitation was not employed, to failure in efficient utilization of the available surface area either as a result of "channelling" within the internal circuit, or inadequate circulation and mixing of the dialyzing fluid. The former possibility was considered the more probable and was supported by direct visualization of channelling following the injection of methylene blue dye into the arterial manifold. This channelling was not observed in the agitated series.

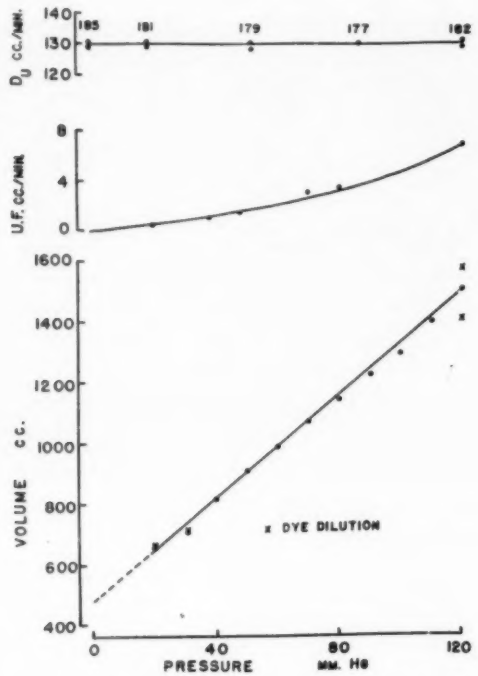

Fig. 5.—The results in one experiment demonstrating the relationships between volume, rate of ultrafiltration (U.F.) and urea dialysance (D_U), and pressure. The volume of the unit was determined at several points both by the dye dilution technique utilizing aqueous solution of hemoglobin, and by the uptake in volume of the unit from a volumetric cylinder. The rate of ultrafiltration was measured by determining the rate of loss from the volumetric cylinder during several consecutive 10- to 30-minute intervals. No solute was present in the circulating fluid during these observations. Urea dialysance was measured at the various pressures during relatively constant flows as indicated by the number above each result.

Dialysance as defined by Wolf and associates[11] was calculated by multiplying the rate of flow through the unit by the ratio of the differences in concentration, between the inflow and outflow, and the inflow and bath solutions. The results for urea are plotted in Fig. 4. Dialysance is clearly flow limited at low flows, whereas there is little increase beyond 350 ml. per minute due to the restriction imparted by the membrane diffusing capacity.

Six experiments were performed in an attempt to measure both the volume of the unit and the rate of ultrafiltration under various conditions of pressure and flow. The pressure was altered by either increasing or decreasing the degree of constriction of the outflow tract, while the flow was controlled by means of a pump. A known quantity of water was pumped from a volumetric cylinder into the unit until it was filled, the efflux then being returned to the source. Consequently, any abrupt change in the volume within the cylinder could be inversely equated to the rapid alteration in the volume of the unit which followed any instantaneous variation in pressure from 0 to 120 mm. Hg and/or flow from 50 to 400 ml. per minute. Following this rapid change, and while pressure and flow were kept constant, any further continuous loss of fluid from the volumetric cylinder was attributed to ultrafiltration. The rate of this loss was measured over several consecutive periods of 10 to 30 minutes' duration. The volume of the entire system was checked at several different pressure levels by the dye dilution technique utilizing hemoglobin in aqueous solution as the indicator. The hemoglobin concentration was determined spectrophotometrically at 550 mμ. The dynamic volume of the unit, including the tubing and manifolds, was calculated to be the difference between the total volume of the system as determined by the degree of dye solution and the volume within the volumetric cylinder.

The data obtained in one representative experiment relating volume to pressure are plotted in Fig. 5. In 3 experiments the minimum volume that was required to fill the unit after the cellulose tubing had been allowed to "damp dry" was 480 to 550 ml. This range was similar to the ordinate intercept obtained by extrapolating the volume/pressure curve to 0 mm. Hg. In Fig. 5, it can be seen that the increment in volume approximated 8 ml. for each millimeter of mercury increase in outflow tract pressure. The range in the other experiments was from 7.9 to 8.8 ml. per mm. Hg. The extent of the gain in the volume over the pressure range of 0 to 120 mm. Hg amounted to 950 to 1,050 ml. From these data, the width of the "blood channel," that is, the residual space between the rod and the membrane, was calculated to vary from 0.5 to 1.7 mm.

The data correlating the rate of ultrafiltration to pressure in the same experiment as mentioned previously are also illustrated in Fig. 5. The results from all the experiments have been listed in Table I, and demonstrate again the expected direct relationship between these factors. In none of these experiments was the rate of ultrafiltration measured in the presence of significant concentrations of solute. In view of this, the actual rate of ultrafiltration could be expected to be less than that indicated for any given hydrostatic pressure when significant unbalanced osmotic forces were present.

The influence of variations in flow rates between 50 to 400 ml. per minute on the volume within the unit and the rate of ultrafiltration were also studied. Such alterations in flow rates when the outflow was unconstricted were accompanied by outflow tract pressures of less than 20 mm. Hg. Under these circumstances, the variation in the volume of the unit did not exceed 35 ml. for each 100 ml. per minute change in flow rate. The rate of ultrafiltration

TABLE I. THE RESULTS IN ALL EXPERIMENTS IN WHICH VARIATIONS IN THE RATE OF ULTRAFILTRATION WERE EFFECTED BY GRADED CONSTRICTION OF THE OUTFLOW TRACT

NUMBER OF OBSERVATIONS	OUTFLOW TRACT PRESSURE (MM. Hg)	AVERAGE RATE OF ULTRAFILTRATION (ML./MIN.)	RANGE RATE OF ULTRAFILTRATION (ML./MIN.)
2*	0	0.09	0.08-0.1
3	20	0.6	0.5-0.7
5	25	0.68	0.5-0.9
3	38	1.0	0.9-1.2
4	50	1.85	1.8-2.0
4	62	2.5	2.4-2.6
7	70	3.6	3.3-3.9
7	80	3.8	3.6-4.0
3	90	5.2	4.5-5.7
7	120	7.5	7.2-8.0

*Periods of 1 hour.

ranged from 0 to 0.7 ml. per minute. However, when the outflow tract was so constricted that a 20 mm. Hg pressure gradient was established at the minimum flow rate, instantaneous changes thereafter in the flow rate induced proportionate pressure alterations within the outflow tract. The variations in the volume of the unit were similar to those induced when equivalent pressures were estabilshed by graded constriction of the outflow tract while the flow rate was constantly maintained. Similarly, no changes in the volume of the unit were observed when, following a change in flow rate, the degree of constriction of the outflow tract was readjusted to maintain a constant pressure within the outflow tract.

In 4 experiments the pressure within the unit was varied while dialysance measurements were being made. In the experiment illustrated in Fig. 5, these observations followed the above-mentioned studies. No attempt has been made to assess the effect of the osmotic activity of the solute on the rate of ultrafiltration. In this experiment, however, the agitation rate was maintained at 136 to 140 per minute between 0 to 30 mm. Hg. Above this pressure, the rate of agitation was reduced to 100 per minute. Under these circumstances, variations in pressure had no discernible effect on urea dialysance. In the 3 other experiments, however, agitation was initially adjusted to 100 to 120 per minute and thereafter so maintained. Increases in dialysance amounted to 4.4 per cent, 6.3 per cent, and 9.4 per cent as the pressure increased from 0 to 30 mm. Hg. An additional increase in dialysance of 2.3 per cent, 7.0 per cent, and 7.8 per cent was observed when the pressure was further raised to 120 mm. Hg. A portion of this increase in dialysance may be accounted for by the increased rate of ultrafiltration. The possible significant role of increased diffusion associated with more turbulence and better mixing within the internal circuit, resulting from either the faster rate of agitation or more effective agitation as the membrane was distended, cannot be excluded.

In Vivo.—Seven experiments were performed on 6 dogs. In 5 of these animals, both ureters were ligated several days prior to hemodialysis. A spontaneous increase in the plasma urea nitrogen and creatinine concentrations ensued, the levels reaching to about 150 and 11 mg. per cent, respectively. Additional urea and creatinine were administered parenterally to 4 animals, 1, 1½, 6, and 18 hours (Experiments 4, 5, 6, and 7) prior to hemodialysis, in

order to raise the urea nitrogen and creatinine concentrations to greater than 250 and 16 mg. per cent, respectively. Following hemodialysis, the ureteral ligatures were released. These 4 animals recovered fully and survived without apparent complications. One animal (Experiment 1) did not undergo release of the bilateral ureteral ligation and succumbed 36 hours after dialysis with a serum potassium of 10 mEq. per liter. Hemodialysis was carried out on two separate occasions in the sixth animal (Experiments 2 and 3), in which no ureteral ligation was performed. Priming doses of urea and creatinine were administered. The urine was collected quantitatively thereafter until the termination of the hemodialysis. The animal recovered fully and survived without complications.

The animals were weighed prior to and usually at the conclusion of most of the experiments. Anesthesia was initiated with sodium pentobarbital, 30 mg. per kilogram and maintained as necessary with small additional doses. Anticoagulation was initially accomplished with heparin, 1.5 mg. per kilogram, followed by 10 mg. per hour. At the conclusion of each experiment, coagulation was restored by the administration of an equivalent amount of hexadimethrine bromide (Polybrene).*

For each of these experiments, the internal circuit of the unit was chemically sterilized with 2 liters of 0.4 per cent formaldehyde solution in 50 per cent autoclaved glycerine. The venous manifold was clamped off after 1,500 ml. of this solution had drained through the unit. The residual solution was retained for at least 12 hours or until the apparatus was prepared for the experiment. Several individual components have been kept for as long as 3 months with this solution without apparent deterioration.

Just prior to the experimental use of the apparatus, the tank (Fig. 2, *A*) was flushed with approximately 300 liters of tap water while the inner circuit was washed out with 10 liters of sterile saline. Aliquots from the final efflux failed to reveal any growth on culture for both bacteria and fungi. The unit was then primed with 500 ml. of 6 per cent dextran in 5 per cent dextrose solution to which 5 mg. of heparin had been added. The venous outflow channel was clamped off so that 200 to 300 ml. of this solution was retained in the unit.

Blood was taken from the femoral artery and returned to the femoral vein after passing through the unit and a combination bubble trap and filter. No accessory pump was utilized except in Experiment 3. All experiments were performed with agitation as previously described. In each experiment, spectrophotometric measurements of the plasma for hemoglobin failed to reveal any significant hemolysis. In two experiments, the venous outflow was measured by a calibrated flow meter. In both of these experiments, the extraction fractions were determined periodically as in the in vitro experiments. In the first of these experiments, the urea and creatinine extraction fractions ranged from 0.47 to 0.38 and 0.37 to 0.27, respectively, at a relatively constant blood flow of 200 ml. per minute. The values for the second experiment, at blood flows of 200 to 240 ml. per minute, ranged from 0.57 to 0.38 for urea and 0.45 to 0.30 for creatinine.

*Polybrene was supplied by the Abbott Laboratories, Chicago, Ill.

The significant data in all 7 experiments are presented in Table II. Both plasma urea nitrogen and creatinine concentrations were considerably reduced by the hemodialysis. The decrease in hematocrit was undoubtedly related in large part to the use of dextran solution to fill the extracorporeal circuit, although some blood loss was continuously sustained in the surgical wounds.

TABLE II. THE PREDIALYSIS AND POSTDIALYSIS RESULTS IN VIVO*

EXPERIMENT	WEIGHT (KG.)		DIALYSIS TIME (MIN.)	PLASMA UREA N (MG. %)		PLASMA CREATININE (MG. %)		HEMATOCRIT (VOL. %)		REMOVED (GM.)	
	PRE-	POST-		PRE-	POST-	PRE-	POST-	PRE-	POST-	UREA N	CREATININE
1	19.0	18.8	240	236	61	16.4	6.7	42	29	17.6	1.3
2†	26.0	26.2	120	120	52	3.6	1.4	40	36	3.9	.24
3†	24.6	24.3	180	141	60	—	—	42	36	14.7	—
4‡	18.2	18.4	300	294	69	16.5	4.0	41	20‡	31.7	1.4
5§	20.0	19.3	300	308	55	15.0	5.5	27§	29	31.3	1.7
6	18.0	—	300	357	77	19.1	6.5	39	30	33.2	2.3
7	26.0	—	240	308	113	17.0	8.3	38	36	26.8	2.2

*Bilateral ureteral ligations were performed in all experiments except 2 and 3. The ligatures were not removed in Experiment 1 following hemodialysis, and the animal died 36 hours later with a serum potassium of 10 mEq. per liter. In the remaining experiments, all the animals survived without complications.

†Experiments 2 and 3 were performed in the same animal at 1-month intervals. Priming doses of 50 Gm. of urea and 460 mg. of creatinine were utilized. In Experiment 2, the urine output was 300 ml. and 3.1 Gm. of urea N for the 30-minute interval between priming and the onset of hemodialysis. During hemodialysis, 250 ml. of urine and 3.4 Gm. of urea N were excreted. In the same intervals, 297 and 160 mg. of creatinine were excreted. In Experiment 3, the urinary losses in the 1-hour interval between the priming dose and the start of the procedure amounted to 6.3 Gm. of urea N in 550 ml. The blood flow through the unit was increased by means of an accessory pump which led to a reduction in the systemic arterial pressure from 110 to 80 mm. Hg. The urine flow decreased to 75 ml. during the procedure with an excretion of 0.77 Gm. of urea N. Through an oversight, the plasma filtrates and tank samples were discarded prior to the creatinine analyses.

‡Estimated loss of 200 ml. of blood from a leaking arterial cannula just after the start of hemodialysis. This was replaced by 6 per cent dextran in 5 per cent dextrose solution.

§Estimated loss of 250 ml. of blood from leaking arterial cannula just prior to start of hemodialysis. This was replaced by 6 per cent dextran in 5 per cent dextrose solution.

DISCUSSION

The data as presented demonstrate the effectiveness of this apparatus in the dialysis of urea and creatinine under in vitro and in vivo experimental conditions. Studies characterizing the properties of the cellulose membrane have shown that other substances will diffuse through the membrane at proportional rates which are in large part determined by molecular structure.[11] Therefore, it is appropriate to assume that these substances will diffuse through the cellulose membrane in this apparatus in a similar manner.

There are several additional features of this new design which are highly advantageous. These include transparency, pretestability, very low resistance to flow, and a fairly wide range in the rate of ultrafiltration.

The present unit, however, incorporates several disadvantages which reduce its over-all usefulness as an ideal clinical tool. These stem from the use of methyl methacrylate which was dictated by its availability and machinability. Its cost is rather high but not prohibitive when purchased in small quantities for disposal after a single use. Furthermore, chemical sterilization, which is more hazardous, is required although this may be obviated as the availability of gas sterilization increases. Other less expensive machinable or formable nontoxic plastics which can be subjected to autoclaving are being investigated in an attempt to overcome these objections.

The extracorporeal volume is not as invariable as that of the externally supported units. Such marked increment in this volume as might occur following a predetermined desire to increase the rate of ultrafiltration, the use of a small venous cannula or unusual venospasm can be anticipated in accordance with the volume/pressure characteristics. These changes can be monitored by measuring the outflow tract pressure. However, the extracorporeal volume under the usual in vivo experimental circumstances is relatively fixed to within acceptable limits. Changes in the rate of blood flow will not cause significant alterations in this extracorporeal volume and therefore can be disregarded.

SUMMARY

The details of design, operation, and the results obtained with a new continuous hemodialyzer are described. The advantages include efficient utilization of the available membrane surface area, transparency, pretestability, low resistance to flow, and a wide range in the rate of ultrafiltration. The extracorporeal volume is, however, dependent upon the outflow tract pressure. At low outflow tract pressures, as is the usual experimental situation in vivo, this extracorporeal volume is within reasonable limits and is relatively fixed.

Methyl methacrylate rods are currently being employed and carry the additional disadvantages of expense and the need for chemical sterilization.

We wish to express our appreciation to Mr. Allan Wilson, Mr. John Stine, and Miss Marion Garner for their technical assistance without which this project could not have been completed.

Addendum.—Dr. W. J. Kolff has called our attention to a paper by himself and Dr. H. T. J. Berk,[12] in which reference is made to a somewhat similarly designed hemodialyzing apparatus constructed by Professor R. Brinkman.

REFERENCES

1. Kolff, W. J., and Berk, H. T. J.: Artificial Kidney Dialyzer With Great Area, Acta med. scandinav. **117**: 121, 1944.
2. Skeggs, L. J., Jr., Leonards, J. R., and Heisler, C. R.: Artificial Kidney. II. Construction and Operation of Improved Continuous Dialyzer, Proc. Soc. Exper. Biol. & Med. **72**: 539, 1949.
3. Alwall, N.: On the Artificial Kidney. I. Apparatus for Dialysis of Blood in vivo, Acta med. scandinav. **128**: 317, 1947.
4. Murray, G., Delorme, E., and Thomas, N.: Development of the Artificial Kidney, Arch. Surg. **55**: 505, 1947.
5. Rosenak, S. S., Oppenheimer, G. D., and Saltzman, A. S.: A Coil Kidney, Exhibition at the American Urological Association, Boston, 1948.
6. Rosenak, S. S., and Saltzman, A. S.: A New Dialyzer for Use as an Artificial Kidney, Proc. Soc. Exper. Biol. & Med. **76**: 471, 1951.
7. Inouye, W. Y., and Engelberg, J.: Simplified Artificial Dialyzer and Ultrafilter. Proc. Surg. Forum, Clin. Congress, Am. Coll. Surgeons, Philadelphia, 1953, W. B. Saunders Company, vol. 4, p. 438.
8. Kolff, W. J., and Watschinger, B.: Further Development of a Coil Kidney; Disposable Artificial Kidney, J. Lab. & Clin. Med. **47**: 969, 1956.
9. Hawk, P. B., Oser, B. L., and Somerson, W. H.: Practical Physiological Chemistry, ed. 13, New York, 1954, McGraw-Hill Book Company, Inc., p. 552.
10. Brod, J., and Sirota, J. H.: The Renal Clearance of Endogenous "Creatinine" in Man, J. Clin. Invest. **27**: 645, 1948.
11. Wolf, A. V., Remp, D. G., Kiley, J. E., and Currie, G. D.: Artificial Kidney Function; Kinetics of Hemodialysis, J. Clin. Invest. **30**: 1062, 1951.
12. Kolff, W. J., and Berk, H. T. J.: Techniek en Chemische Resultatent der Vividialyse, Arch. Néerl. de physiol. **28**: 166, 1947.

THE INTERRELATIONSHIP OF WEDGED HEPATIC VEIN PRESSURE, INTRASPLENIC PRESSURE, AND INTRA-ABDOMINAL PRESSURE

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DURING the past decade, increasing interest in the surgical therapy of portal hypertension has stimulated the development of methods to quantitate portal pressure. The direct measurement of portal vein pressure in patients during celiotomy has been carried out for many years. However, this procedure does not lend itself to the systematic study of portal hypertension, and recent attention has been directed at indirect measurements of portal venous pressure. In 1951, Myers and Taylor[1] showed that pressure readings recorded from a cardiac catheter wedged in a hepatic vein reflected portal pressure. This observation was soon confirmed and wedged hepatic vein pressures were recorded in liver disease,[2] portal vein abnormalities,[3] under anesthesia, and celiotomy,[4] and under the influence of various pharmacologic stimuli.[5]

Percutaneous splenic puncture and measurement of the splenic pulp pressure have also been found to reflect portal pressure.[6] Although this procedure is not without risk, it is an easier technique than that necessary to obtain wedged hepatic vein pressure, which requires catheterization through the right side of the heart. Other methods of estimating portal vein pressure have included measurement of the pressure in various collaterals, for example, esophageal varices[7] and anterior abdominal wall veins.[8]

Atkinson and Sherlock[6] compared intrasplenic pressures to subsequent measurements of wedged hepatic vein pressure, and to direct portal vein pressure measured at operation. They found a close relationship, in the absence of extrahepatic portal vein obstruction, between intrasplenic and portal vein pressure, if the latter were either measured directly or by occluding a hepatic vein. Although they excluded patients with ascites from their series, they suggested that a rise in intra-abdominal pressure would increase the portal venous pressure and would be reflected in both the splenic pulp pressure and the wedged pressure. It is possible that this may have therapeutic implications. In patients with ascites associated with bleeding due to portal hypertension, some reduction in portal pressure may be accomplished by abdominal paracentesis.

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In order to assess this relationship, experiments were performed in dogs to quantitate the effect of artificial variation in intra-abdominal pressure upon these clinical parameters of portal pressure.

MATERIALS AND METHODS

In six adult mongrel dogs, weighing 10 to 15 kilograms, the following plan was carried out. Initially, the dogs were subjected to laparotomy and transplantation of the tip of the spleen into a subcutaneous pocket, without alteration of its vascular attachments.[9] After the tip of the spleen becomes fixed in its new site, percutaneous puncture can be performed without difficulty. When this had occurred, the animals were anesthetized for study. A cardiac catheter was inserted through an external jugular vein, and under fluoroscopic control, was guided into a hepatic vein, and "wedged" into position. In four of the animals, another catheter was positioned in the abdominal inferior vena cava after insertion through a femoral vein. Percutaneous splenic puncture was performed with a thin-walled, 17 gauge needle. Pressures were obtained from these sites using Statham strain gauges, and permanent tracings were obtained on a multichannel recorder. A trocar was inserted into the peritoneal cavity through

Fig. 1.—Roentgenogram of the abdomen showing catheters and intrasplenic needle positioned for simultaneous pressure measurements.

the right lower quadrant, and a cardiac catheter was threaded through it. This catheter was used to monitor intra-abdominal presssure and to infuse isotonic saline into the peritoneal cavity. When all the catheters were in position (Fig. 1), base line pressures were recorded with the animal supine; a point midway between the sternum and the fluoroscopy table was used as zero. Saline was then infused rapidly into the abdominal cavity until the animal had tense "ascites," as manifested by respiratory distress. Simultaneous pressures were recorded with each 500 ml. increment in the abdominal fluid. Since there were variations in the pressures with respiration, the values described below were measured during the expiratory phase of respiration.

RESULTS

The findings were of the same general character for all animals studied. Fig. 2 illustrates the changes observed in the various parameters during a typical experiment (Dog 837).

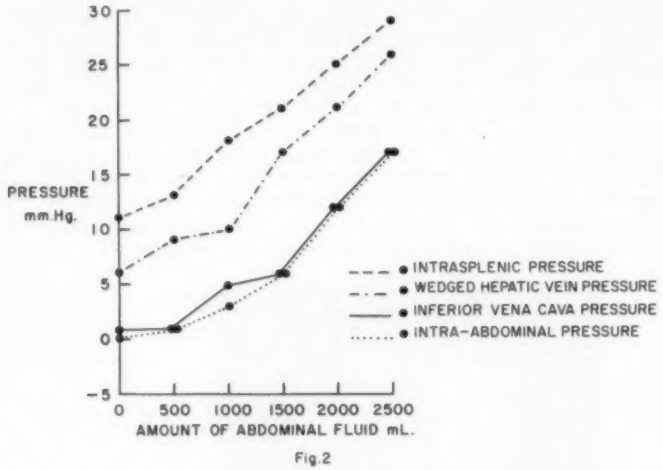

Fig. 2.—Changes observed in intrasplenic, wedged hepatic vein, inferior vena cava, and intra-abdominal pressures during a typical experiment (Dog 837).

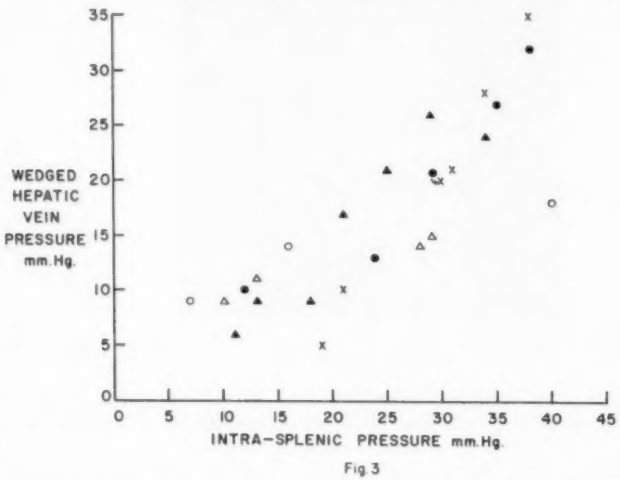

Fig. 3.—Comparison of the wedged hepatic vein pressure and the intrasplenic pressure with increasing intra-abdominal pressure.

Intra-abdominal pressure rose slowly during the saline infusion. The degree of rise was, in general, proportional to each 500 ml. increment in abdominal fluid. However, a more rapid increase occurred as a tense abdomen

was attained. The actual amount of fluid necessary to produce tense "ascites" varied from animal to animal, depending upon the resistance afforded by the abdominal musculature. The range was 2,000 to 3,000 ml. of saline infused, resulting in a mean rise in intra-abdominal pressure from 0 to 17 mm. of mercury.

Intrasplenic pressure rose rapidly with small increments in intra-abdominal pressure and reached the highest levels of all the parameters measured. Initially,

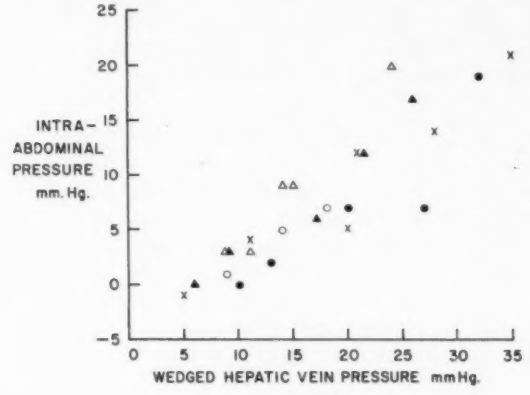

Fig. 4

Fig. 4.—Changes in the wedged hepatic vein pressure with increasing intra-abdominal pressure.

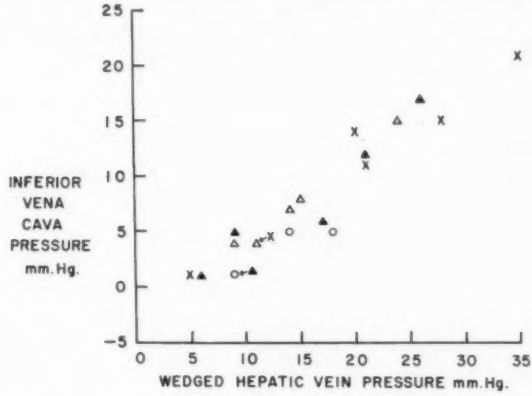

Fig. 5

Fig. 5.—Comparison of the wedged hepatic vein pressure and the inferior vena cava pressure with increasing intra-abdominal pressure.

minimal increases in intra-abdominal pressure caused a disproportionate rise in the intrasplenic pressure. On the other hand, as the abdomen became tense, intra-abdominal pressure increased more rapidly than the splenic pressure. The mean base line splenic pressure was 12 mm. of mercury, and the mean maximum pressure attained was 33 mm. of mercury.

The wedged hepatic vein pressure paralleled in a general way the rising intrasplenic pressure at a lower level. The wedged pressure correlated with the splenic pressure, r (correlation coefficient) being 0.85 (Fig. 3). The wedged pressure correlated somewhat more closely with the increasing intra-abdominal pressure, r being 0.89 (Fig. 4). An increment in intra-abdominal pressure resulted in a proportional rise in the wedged pressure. Similarly, the wedged pressure correlated closely with the inferior vena cava pressure (Fig. 5). The mean base line wedged pressure was 9 mm. of mercury and the mean maximum pressure reached was 24 ml. of mercury.

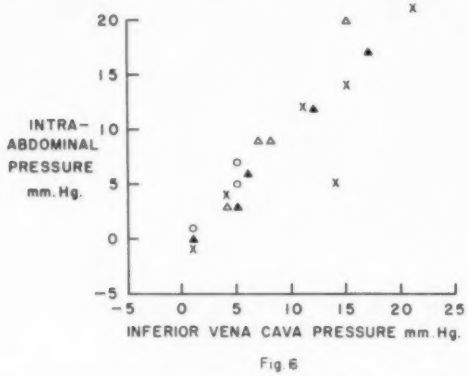

Fig. 6

Fig. 6.—Changes in the inferior vena cava pressure with increasing intra-abdominal pressure.

In those experiments where the inferior vena cava pressure was monitored, it was almost identical to the intra-abdominal pressure (Fig. 6).

DISCUSSION

These results confirm the suggestion of Atkinson and Sherlock that intra-abdominal pressure affects portal pressure, as measured here by wedged hepatic vein pressure and intrasplenic pressure. Reynolds,[10] Krook,[11] and others have corrected for this effect by subtracting inferior vena cava pressure from observed wedged pressure, in order to estimate portal pressure more accurately in the presence of ascites. Reynolds has measured wedged pressure and caval pressure simultaneously before and after paracentesis. He found that the absolute values fell proportionately with drainage of the ascites, so that the gradient between the 2 pressures remained constant. The present study further substantiates the practice of correcting wedged hepatic vein pressure by subtracting inferior vena cava pressure, since the rising wedged pressure paralleled the increasing intra-abdominal and caval pressures. It should be emphasized that the present experiments have demonstrated this relationship to be valid in the absence of the abnormal vasculature that is present in cirrhosis. These findings, however, are in agreement with Reynolds' observations of cirrhotic patients and there is no reason to assume that the presence of cirrhosis would have altered these results.

Our observations indicate that all determinations of wedged pressure should be corrected by caval pressure, since small amounts of abdominal fluid, possibly undetected, can affect portal pressure. It should be pointed out, however, that the effective pressure in an esophageal varix in a patient with portal hypertension and ascites is not this "corrected" pressure, but actually the total pressure, including the increment contributed by the increased intra-abdominal pressure. Therefore, it would seem logical that abdominal paracentesis should be considered in the therapy of bleeding in such a patient, to eliminate the effect of the intra-abdominal pressure. It is well known that paracentesis, per se, in a cirrhotic patient may precipitate hepatic coma and death. Thus, the advantage to be gained from this treatment must be weighed carefully before it is undertaken.

Wedged hepatic vein pressure was found to correlate with the intrasplenic pressure. This discovery confirmed previous findings concerning patients with liver disease.[6] In spite of this reasonably good degree of correlation, however, intrasplenic pressure responded differently than the wedged pressure to the increasing intra-abdominal pressure. The disproportionate rise in splenic pressure noted with the initial, minimal increments in intra-abdominal pressure may have been caused by the artificial position of the transplanted spleen. Therefore, in this series of experiments, pressures recorded from the splenic pulp are valid in reflecting the general trend, but should not be viewed in terms of absolute values.

SUMMARY AND CONCLUSIONS

1. The importance of indirect measurements of portal venous pressure and the validity with which the most frequently used clinical parameters (wedged hepatic vein pressure and intrasplenic pressure) reflect portal pressure have been briefly reviewed.

2. Ascites was artificially induced in dogs, while simultaneous pressures were recorded, to study the relationship of these clinical parameters to increasing intra-abdominal pressure.

3. Increments in the intra-abdominal pressure were found to be reflected in the wedged hepatic vein pressure as a parallel rise in pressure.

4. Although the intrasplenic pressure correlated with the wedged hepatic vein pressure, its relationship to the intra-abdominal pressure was proportional only in a general way.

5. The importance of these results in estimating the true wedged hepatic vein pressure in the presence of small amounts of abdominal fluid has been discussed.

6. It has been suggested that when ascites is associated with portal hypertension and bleeding esophageal varices, cautious abdominal paracentesis may be a useful therapeutic measure for it reduces intra-abdominal pressure and, thereby, lowers portal pressure.

REFERENCES

1. Myers, J. D., and Taylor, W. J.: An Estimation of Portal Venous Pressure by Occlusive Catheterization of an Hepatic Venule, J. Clin. Invest. 30: 662-663, 1951.

2. Krook, H.: Estimation of Portal Venous Pressure by Occlusive Hepatic Vein Catheterization, Scandanav. J. Clin. & Lab. Invest. **5**: 285-292, 1953.
3. Paton, A., Reynolds, T. B., and Sherlock, S.: Assessment of Portal Venous Hypertension by Catheterization of Hepatic Vein, Lancet **1**: 918-921, 1953.
4. Reynolds, T. B., Balfour, D. C., Jr., Levinson, D. C., Mikkelsen, P. W., and Pattison, A. C.: Comparison of Wedged Hepatic Vein Pressure With Portal Vein Pressure in Human Subjects With Cirrhosis, J. Clin. Invest. **34**: 213-218, 1955.
5. Friedman, E. W., and Weiner, R. S.: Estimation of Hepatic Sinusoid Pressure by Means of Venous Catheters and Estimation of Portal Pressure by Hepatic Vein Catheterization, Am. J. Physiol. **165**: 527-531, 1951.
6. Atkinson, M., and Sherlock, S.: Intrasplenic Pressure as an Index of Portal Venous Pressure, Lancet **1**: 1325-1327, 1954.
7. Allison, P.: The Measurement of Blood Pressure in Oesophageal Varices, Thorax **6**: 325-327, 1951.
8. Davidson, C. S., Gibbons, T. B., and Faloon, W. W.: Systemic and Portal Venous Pressures in Cirrhosis of the Liver, J. LAB. & CLIN. MED. **35**: 181-187, 1950.
9. Davis, R. E., Hallenbeck, G. A., Lichtenheld, F. R., and Grindlay, J. H.: Percutaneous Splenic Portograms in Dogs: Technique and Examples of Usefulness, Surgery **38**: 708-711, 1955.
10. Reynolds, T. B., Redeker, A. G., and Geller, H. M.: Wedged Hepatic Venous Pressure: A Clinical Evaluation, Am. J. Med. **22**: 341-350, 1957.
11. Krook, H.: Circulatory Studies in Liver Cirrhosis, Acta med. Scandinav. Suppl. 318, 1957.

LABORATORY METHODS

THE FLUOROMETRIC ESTIMATION OF MAGNESIUM IN SERUM AND IN URINE

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SEVERAL investigators have used 8-hydroxyquinoline for estimating magnesium in biologic fluids. The methods described involve the complete precipitation of magnesium 8-hydroxyquinoline from hot ammoniacal solution, followed by gravimetric,[1] colorimetric,[2, 3] titrimetric,[4] or flame photometric[5] estimation of the precipitate. The present report describes a procedure with 8-hydroxyquinoline which is simpler and does not involve a precipitation step. It is based on the observation that ethanolic solutions of magnesium 8-hydroxyquinoline exhibit a characteristic fluorescence when activated by light of wave length 420 mμ. This property has been utilized to develop a sensitive, relatively specific, accurate, and rapid method for estimating the magnesium in serum and in urine.

MATERIALS AND METHODS

Reagents.—Reference standard solutions of $MgCl_2$ were prepared from reagent grade MgO (Matheson Company) dried for 24 hours at 110° C., dissolved in a small volume of concentrated HCl, and diluted to volume with deionized water. A solution containing 5 Gm. of 8-hydroxyquinoline (certified reagent, Fisher Scientific Company) in 100 ml. of absolute ethanol was freshly prepared every two days and stored at 5° C. in a brown bottle. The pH values of all buffer solutions were checked with the Beckman glass electrode pH meter.

Fluorescence.—Measurements of fluorescence were made with the Aminco-Bowman spectrophotofluorometer[6] and expressed in arbitrary units. Solutions of $MgCl_2$ in 80 per cent ethanol treated with an excess of 8-hydroxyquinoline exhibit fluorescence with a peak intensity at 530 mμ when activated at the optimal wave length, 420 mμ.* Fig. 1 illustrates that the intensity of fluorescence is dependent on pH and the buffer used, with maximal fluorescence at a pH of approximately 9.5.

In the presence of 0.1 M sodium acetate buffer, the fluorescence of magnesium hydroxyquinoline is minimal from pH 3.5 to 5.0 and increases rapidly from pH 5.0 to 6.5 (Fig. 1, curve A); similar solutions containing calcium instead of magnesium exhibit negligible fluorescence throughout this range of pH values (Fig. 1, curve B). Fig. 2 shows that the increment in fluorescence from pH 3.5 to 6.5 is directly proportional to the magnesium concentration from 0.025 to 0.200 mEq. per liter and, therefore, is suitable for the estimation of magnesium in serum and urine. Solutions containing calcium instead of magnesium do not exhibit increments in fluorescence (Fig. 2).

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*The maximal fluorescence at 450 mμ of quinine sulfate in 0.1 N H_2SO_4 was observed with an activating wave length of 360 mμ with the spectrophotofluorometer used in this work.

TABLE I. THE FLUORESCENCE OF MAGNESIUM 8-HYDROXYQUINOLINE SOLUTIONS AT VARIOUS CONCENTRATIONS OF ETHANOL OR SODIUM ACETATE BUFFER*

CONCENTRATION OF ETHANOL % (V/V)	Δ (+) FLUORESCENCE†	CONCENTRATION OF SODIUM ACETATE BUFFER (M)	Δ (+) FLUORESCENCE†
92.5	12.3	0.05	6.3
80.0	5.7	0.10	5.7
67.5	2.0	0.15	5.2
55.0	1.0	0.20	4.8
25.0	0.2	0.25	4.5

*The standard systems containing sodium acetate buffer of pH 3.5 or 6.5 are described in the legends to Figs. 1 and 2. The final concentrations of ethanol and sodium acetate buffer were varied as indicated above, and the fluorescence at 530 mμ was measured with an activating wave length of 420 mμ.

†Fluorescence at pH 6.5 minus fluorescence at pH 3.5.

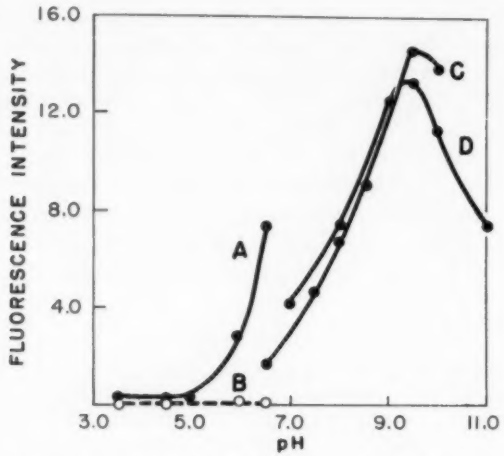

Fig. 1.—Fluorescence at 530 mμ of ethanolic solutions of magnesium (●——●) or calcium (○- - -○) 8-hydroxyquinoline at various pH values. The activating wave length was 420 mμ. The solutions tested were prepared by mixing 0.2 ml. of 5×10^{-4} M $MgCl_2$ (or $CaCl_2$), 0.1 ml. of 5 per cent 8-hydroxyquinoline in ethanol, 1.5 ml. of absolute ethanol, 0.1 ml. of deionized water, and 0.1 ml. of one of the following buffer solutions at the appropriate pH: 2.0 M sodium acetate (curves A and B), 0.4 M glycine (curve D), 2.0 M tris(hydroxymethyl)-aminomethane (curve C). In each instance the fluorescence of a control solution containing all the constituents minus $MgCl_2$ or $CaCl_2$ was deducted from the fluorescence observed with the complete system.

An increase in the concentration of water or of sodium acetate buffer quenches the magnesium hydroxyquinoline fluorescence at pH 6.5 as indicated in Table I. The addition of successive quantities of sodium Versenate (ethylenediaminetetraacetate) to chelate the magnesium apparently dissociates the magnesium hydroxyquinoline complex, inasmuch as the intensity of the fluorescence is progressively diminished (Fig. 3). Complete suppression of the fluorescence is observed with a molar ratio of Versenate to magnesium of approximately 1.25.

Other Cations.—A number of other cations were tested for their capacity to yield fluorescent solutions in 80 per cent ethanol when treated with 8-hydroxyquinoline. Each cation was tested at pH 3.5 and 6.5 in the presence of 0.1 M sodium acetate buffer. As shown in Table II only zinc solutions demonstrate a significant increment in fluorescence (pH 6.5 minus pH 3.5) and this increment is approximately one half that observed with magnesium solutions. Inasmuch as the zinc concentration in human serum[7] and urine[8] is approximately

2 to 3 per cent of the magnesium concentration, the contribution of the zinc fluorescence is negligible (i.e., less than 1.5 per cent) in the fluorometric estimation of magnesium in these fluids.

Estimation of Magnesium in Serum.—The estimations are performed in duplicate with the following two reagents prepared just before use. Reagent 1 (pH 3.5) is prepared by mixing 2 volumes of 2.0 M sodium acetate buffer of pH 3.5, 2 volumes of 5 per cent 8-hydroxyquinoline in ethanol, 30 volumes of absolute ethanol, and 5 volumes of deionized water. Reagent 2 (pH 6.5) is prepared in an identical manner except that 2.0 M sodium acetate buffer of pH 6.5 replaces the pH 3.5 buffer. For each estimation 3.9 ml. of reagents

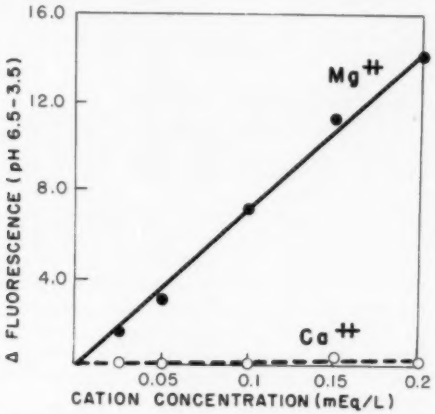

Fig. 2.—Increment in fluorescence (fluorescence at pH 6.5 minus fluorescence at pH 3.5) observed with various concentrations of $MgCl_2$ (●——●) or $CaCl_2$ (○- - -○). The solutions were prepared with the 2.0 M sodium acetate buffers and tested for fluorescence as described in the legend to Fig. 1. The solutions had a final volume of 2.0 ml. of 80 per cent (v/v) ethanol, and they contained the final concentrations of magnesium or calcium indicated on the horizontal axis. Each concentration of cation was tested with the pH 6.5 and the pH 3.5 buffer, and the increment in fluorescence was calculated.

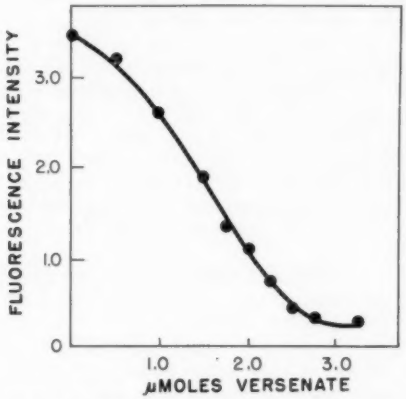

Fig. 3.—Effect of sodium Versenate on the fluorescence of an ethanolic solution of magnesium 8-hydroxyquinoline (activating wave length 420 mμ, fluorescence wave length 530 mμ). The initial solution contained 2.0 μM of $MgCl_2$, sodium acetate buffer of pH 6.5 in a final concentration of 0.1 M, and 8-hydroxyquinoline in a final concentration of 0.017 M in 80.0 ml. of 80 per cent (v/v) ethanol. Successive aliquots of 0.001 M sodium Versenate (aqueous) were added.

TABLE II. FLUORESCENCE OF ETHANOLIC SOLUTIONS OF VARIOUS DIVALENT CATIONS TREATED WITH 8-HYDROXYQUINOLINE*

CATION	FLUORESCENCE (ARBITRARY UNITS)		
	pH 3.5	pH 6.5	Δ (pH 6.5 MINUS pH 3.5)
Mg^{++}	1.2	20.0	+18.8
Zn^{++}	6.6	16.2	+ 9.6
Sr^{++}	0.4	0.5	+ 0.1
Ba^{++}	0.6	0.4	- 0.1
Co^{++}	0.3	0.1	- 0.2
Mn^{++}	0.5	0.1	- 0.4
Cu^{++}	0.3	0.2	- 0.1
Ni^{++}	0.4	0.1	- 0.3
Hg^{++}	0.7	0.3	- 0.4
Fe^{++}	0.2	0.1	- 0.1
Pb^{++}	1.3	1.0	- 0.3

*The solutions tested had a final volume of 4.0 ml. of 80 per cent (v/v) ethanol and contained the following constituents in the final concentrations indicated: divalent cation, 2.5×10^{-4} M; sodium acetate buffer of pH 3.5 or 6.5, 0.1 M; and 8-hydroxyquinoline, 0.017 M. The cations were added as their chloride salts except for $MnSO_4$ and $FeSO_4$. The activating and fluorescence wave lengths were 420 and 530 mμ respectively.

1 and 2, respectively, are transferred to separate, glass-stoppered centrifuge tubes. An aliquot of 0.1 ml. of serum is added to each of the reagents, the tubes are stoppered, shaken vigorously by hand for 2 minutes, and subsequently centrifuged for 20 minutes in a clinical centrifuge. The clear supernatant solutions are decanted directly into cuvettes, and the fluorescence of each is measured with the spectrophotofluorometer (activating wave length 420 mμ, fluorescence wave length 530 mμ). The increment in fluorescence between pH 3.5 and 6.5 is compared with the corresponding increment observed with a reference standard, 0.1 ml. of 0.001 M $MgCl_2$ added to 3.9 ml. of reagents 1 and 2, respectively. Reagent blanks containing water in place of serum exhibit negligible fluorescence.

Five serum samples were tested for magnesium before and after the addition of 20 μM of sodium Versenate per milliliter to test for fluorescence owing to substances other than magnesium. Treatment with Versenate completely abolished the increment in fluorescence (pH 3.5 to 6.5) in each instance, indicating that there is no significant error due to nonspecific fluorescence.

Estimation of Magnesium in Urine.—The procedure for urine is similar to that for serum except that the buffering capacity of the reagents is increased. Reagents 1 (pH 3.5) and 2 (pH 6.5) are prepared just before use by mixing 5 volumes of 2.0 M sodium acetate buffer (of pH 3.5 or 6.5), 2 volumes of 5 per cent 8-hydroxyquinoline in ethanol, and 31 volumes of absolute ethanol. Urine samples collected from normal subjects are diluted 1:5. An aliquot of 0.2 ml. of the urine dilution is added to 3.8 ml. of reagent 1 and to 3.8 ml. of reagent 2. Each solution is mixed, transferred to a cuvette, and the fluorescence measured with the spectrophotofluorometer as described for serum samples. The increment in fluorescence between pH 3.5 and 6.5 is compared with that observed with a reference standard—0.2 ml. of 0.001 M $MgCl_2$ added to 3.8 ml. of reagents 1 and 2, respectively. Centrifugation has not been necessary for estimations of urine samples from normal subjects but may be required if the urine contains protein.

Urine samples from 4 normal subjects were tested for magnesium before and after the addition of 40 μM of sodium Versenate per milliliter of urine. Treatment with Versenate diminished the increment in fluorescence (pH 3.5 to 6.5) by 90.0 to 90.9 per cent. Thus, approximately 10 per cent of the increment in fluorescence observed with these urine samples was apparently not due to magnesium. The urine procedure is easily modified to correct for this nonspecific fluorescence. One aliquot of a urine sample is diluted 1:5 with water and another aliquot is diluted 1:5 with 0.01 M sodium Versenate. Both urine dilutions are then carried through the procedure for magnesium estimation described above. The small increment in fluorescence observed in the presence of Versenate is subtracted from the increment in fluorescence observed without Versenate.

TABLE III. RECOVERIES OF MAGNESIUM ADDED TO SERUM AND TO URINE*

SERUM			URINE		
MAGNESIUM ADDED (MEQ./L.)	MAGNESIUM RECOVERED (MEQ./L.)	RECOVERY (PER CENT)	MAGNESIUM ADDED (MEQ./L.)	MAGNESIUM RECOVERED (MEQ./L.)	RECOVERY (PER CENT)
2.0	2.1	105.0	5.0	4.9	97.0
4.0	4.2	104.5	10.0	9.9	98.5
6.0	6.2	103.2	15.0	15.6	104.0
8.0	7.6	94.4	20.0	20.8	104.0
10.0	9.9	99.0	25.0	24.4	97.5
Mean		101.0	Mean		100.2

*The indicated amounts of $MgCl_2$ were added to a sample of serum and of urine which contained 2.10 and 1.80 mEq. per liter of magnesium, respectively.

RESULTS AND DISCUSSION

Recoveries and Reproducibility.—Varying quantities of magnesium were added to serum and to urine, and the samples were then estimated fluorometrically. As listed in Table III the recoveries of magnesium from serum ranged from 94.4 to 105.0 (mean 101.0) per cent, and the recoveries from urine ranged from 97.0 to 104.0 (mean 100.2) per cent. In a series of duplicate estimations of 9 serum and 5 urine samples, the maximal and average differences between duplicates were 12.5 and 6.9 per cent, respectively, for the serum samples, and 6.3 and 1.3 per cent, respectively, for the urine samples.

Magnesium in Normal Serum.—Venous blood samples were collected from 33 normal, nonfasting, female student nurses aged 18 to 29 years. The samples were allowed to clot for one hour at room temperature, the sera were separated by centrifugation, and the magnesium was estimated fluorometrically. The range of values, 1.65 to 2.44 mEq. per liter, and the mean (± standard deviation), 2.05 ± 0.22 mEq. per liter, agree closely with the results reported recently by investigators using the molybdivanadate,[9] titan yellow,[10] and flame photometric[11] methods (Table IV).

The fluorometric estimation offers several advantages over the other methods listed in Table IV. Unlike the molybdivanadate technique, the fluorometric method does not require the quantitative precipitation of magnesium and, therefore, is a simpler and more rapid procedure. The fluorometric estimation is more sensitive than the titan yellow or flame photometric methods.[10, 12] The procedure described in this report is sufficiently sensitive to estimate as little as 0.1 to 0.2 μg of magnesium in a final volume of 1.0 ml. Thus as little as 10 μl of serum could be used for a single estimation.

TABLE IV. SERUM MAGNESIUM VALUES IN NORMAL SUBJECTS

METHOD	NUMBER OF SUBJECTS	SERUM MAGNESIUM* (MEQ./L.)	REFERENCE NO.
Titan yellow	45	1.87 ± 0.12	10
Molybdivanadate	44	1.95 ± 0.21	9
Flame photometer	14	2.05 ± 0.18	11
Fluorometric	33	2.05 ± 0.22	

*Mean ± standard deviation. The standard deviation for the values obtained by Orange and Rhein[10] was estimated above as one-fourth the range of values.

SUMMARY

Ethanolic solutions of magnesium 8-hydroxyquinoline exhibit fluorescence with a peak intensity at 530 mμ when activated by light at 420 mμ. This property has been utilized to develop a sensitive, relatively specific, accurate, and rapid method for the estimation of magnesium in serum and in urine.

REFERENCES

1. Hahn, F.: Fortschritte in der Analytischen Chemie der Leichtmetalle, Ztschr. angew. Chem. **39**: 1198, 1926.
2. Yoshimatsu, S.: A New Rapid Method for the Determination of Magnesium With One C.C. of Blood, Without Preliminary Elimination of Calcium, Tohoku J. Exper. Med. **14**: 29-35, 1929.
3. Hoffman, W. S.: A Colorimetric Method for the Determination of Serum Magnesium Based on the Hydroxyquinoline Precipitation, J. Biol. Chem. **118**: 37-45, 1937.
4. Greenberg, D. M., and Mackey, M. A.: The Determination of Magnesium in Blood With 8-Hydroxyquinoline, J. Biol. Chem. **96**: 419-429, 1932.
5. Davis, S.: A Flame Photometric Method for the Determination of Plasma Magnesium After Hydroxyquinoline Precipitation, J. Biol. Chem. **216**: 643-651, 1955.
6. Bowman, R. L., Caulfield, P. A., and Udenfriend, S.: Spectrophotofluorometric Assay in the Visible and Ultraviolet, Science **122**: 32-33, 1955.
7. Vallee, B. L., Wacker, W. E. C., Bartholomay, A. F., and Robin, E. D.: Zinc Metabolism in Hepatic Dysfunction. I. Serum Zinc Concentrations in Laennec's Cirrhosis and Their Validation by Sequential Analysis, New England J. Med. **255**: 403-408, 1956.
8. Vallee, B. L., Wacker, W. E. C., Bartholomay, A. F., and Hoch, F. L.: Zinc Metabolism in Hepatic Dysfunction. II. Correlation of Metabolic Patterns With Biochemical Findings, New England J. Med. **257**: 1055-1065, 1957.
9. Stutzman, F. L., and Amatuzio, D. S.: A Study of Serum and Spinal Fluid Calcium and Magnesium in Normal Humans, Arch. Biochem. **39**: 271-275, 1952.
10. Orange, M., and Rhein, H. C.: Microestimation of Magnesium in Body Fluids, J. Biol. Chem. **189**: 379-386, 1951.
11. Wacker, W. E. C., and Vallee, B. L.: A Study of Magnesium Metabolism in Acute Renal Failure Employing a Multichannel Flame Spectrometer, New England J. Med. **257**: 1254-1262, 1957.
12. Vallee, B. L., and Margoshes, M.: Instrumentation and Principles of Flame Spectrometry. Multichannel Flame Spectrometer, Anal. Chem. **28**: 175-179, 1956.

A SIMPLE FLUOROMETRIC PROCEDURE FOR DETERMINING SALICYLIC ACID IN BIOLOGIC TISSUES

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MANY methods for estimating salicylate in plasma have been previously described.[1-5] However, to our knowledge, none of them is sufficiently sensitive or specific for estimating minute amounts of salicylate in various biologic tissues. Although Smith and associates[4] used the method of Brodie and co-workers[1] to measure salicylate in tissues, the procedure is not sensitive enough for measuring the minute quantities found in tissues even when large amounts of the drug are administered.

The procedure for measuring salicylic acid in tissues described in this report involves a separation of the salicylic acid from tissues by extraction into ether, its return to borate buffer, and the subsequent measurement of its fluorescence in the spectrophotofluorometer.[6]

MATERIALS AND METHODS

Reagents.—1. Standard salicylic acid solution, 116 mg. of sodium salicylate is dissolved in a final volume of 100 ml. of water. This standard is stable when stored at refrigerator temperature. Working standards are prepared by dilution with water or 0.5 M borate buffer, pH 10.0.

2. Washed ether: This is prepared by washing commercially available anhydrous ether with an equal volume of 0.1 N NaOH, then 0.1 N HCl and finally with distilled water until the washings are neutral to indicator paper.

3. Hydrochloric acid, 6 N.

4. Borate buffer, 0.5 M, pH 10.0: 35 ml. of 10 N NaOH are added to 31.4 Gm. H_3BO_3 and the volume made up to 1 liter.

Procedure.—Tissues (1 Gm.) are homogenized in 0.01 N HCl (3 ml.), centrifuged at 10,000 g and supernatants taken for analysis. Plasma is diluted to 25 per cent by adding 1 ml. of plasma to 3 ml. of 0.01 N HCl. To a 30 ml. glass-stoppered centrifuge tube add 0.5 ml. of tissue supernatant or diluted plasma, 1 ml. of water and 1 ml. of 6 N HCl and mix. Add exactly 20 ml. of washed ether to the mixture, shake vigorously, and centrifuge at moderate speed for 5 minutes. Transfer exactly 10 ml. of the ether layer to another 30 ml. shaking tube containing 3 ml. of borate buffer, pH 10.0, and shake vigorously. Centrifuge for 5 minutes at moderate speed, then aspirate off the ether layer. Place tubes in a water bath at 55° C. for 5 minutes to evaporate residual ether and cool to room temperature. Transfer 1 ml. of the borate buffer to a cuvette and measure fluorescence in the spectrophotofluorometer with activation set at 310 mμ and fluorescence at 400 mμ.

The accuracy of the Aminco-Bowman spectrophotofluorometer for assaying salicylic acid is best at low concentrations with 2 μg being maximum. Above this amount the

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response is not linear. In tissues with higher concentrations of salicylate, it has been found best to dilute the final aliquot of buffer extract with fresh borate buffer.

Fluorometric readings of standards taken through the extraction procedure indicated that no detectable amount of salicylate is lost during the extraction procedure other than by the aliquots taken. However, as a precaution, standards are prepared by carrying known amounts of salicylate through the entire extraction procedure.

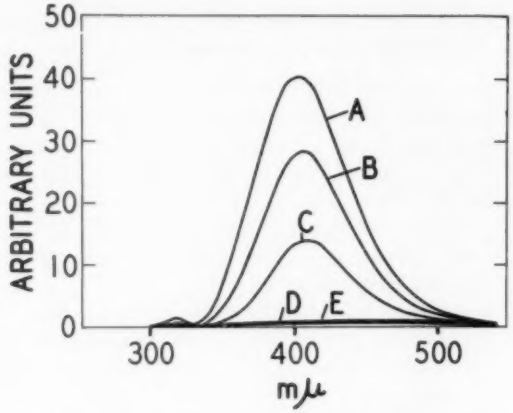

Fig. 1.—Curve A, absolute standard, 2 μg per milliliter; Curve B and C, experimental plasma; Curve D, control plasma; Curve E, blank.

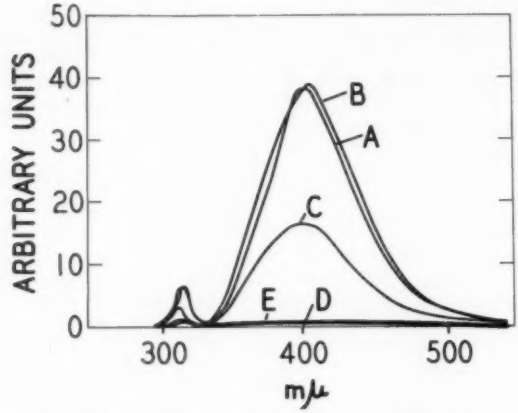

Fig. 2.—Curve A, absolute standard, 2 μg per milliliter; Curve B and C, experimental brain; Curve D, control brain; Curve E, blank.

RESULTS

Specificity.—Activation and fluorescence spectra were determined on salicylate standards, reagent blanks, and experimental tissue extracts. Typical spectra are shown in Figs. 1 and 2.

The absence of detectable blanks in salicylate-free tissues indicates that the constituents of normal tissues do not interfere with the fluorescence assay. Of

TABLE I. RECOVERY OF SALICYLIC ACID ADDED TO TISSUES

TISSUE ASSAYED	SALICYLIC ACID ADDED (μg)					
	6	12	30	90	150	300
Plasma—salicylic acid found	6	12	29	84	144	300
per cent recovery	100	100	97	93	96	100
Brain—salicylic acid found	6	12	31	85	147	300
per cent recovery	100	100	103	94	98	100
Liver—salicylic acid found	6	12	31	86	146	300
per cent recovery	100	100	103	96	97	100
Lung—salicylic acid found	6	12	30	87	147	300
per cent recovery	100	100	100	97	98	100
Kidney—salicylic acid found	6	12	30	87	153	300
per cent recovery	100	100	100	97	102	100

Various amounts of salicylic acid were added to rat tissues (25 per cent) and were extracted and assayed by the described procedure.

the metabolic products of salicylic acid, gentisic acid also exhibits weak fluorescence. However, this occurs at 440 mμ when activated at 315 mμ. A solution containing 2 μg each of salicylic acid and gentisic in borate buffer was found to exhibit exactly the same fluorescence as that of pure salicylic acid with activation set at 310 mμ and fluorescence at 400 mμ.

Recovery From Tissues.—Estimations of salicylic acid added in various amounts to rat tissues were performed to test the accuracy of the method. The data in Table I, where each value is the average of triplicate assays, indicate that salicylic acid added to tissues is recoverable with satisfactory precision.

Disposition of Salicylic Acid in Animals.—In order to ascertain the length of time salicylate remains in plasma, brain, and kidney after administration of sodium salicylate to animals, the following experiment was carried out. Sodium salicylate, 232 mg./Kg., was administered to rats (male, Sprague-Dawley rats of the NIH strain and weighing 240 to 250 grams), and at intervals several animals were sacrificed and the concentration of salicylate in plasma, brain, and kidney was determined. The results, shown in Table II, indicate that even after 24 hours appreciable amounts of salicylate are present in all the tissues studied.

TABLE II. TIME-RATE STUDY OF SALICYLIC ACID IN TISSUES

TISSUE ASSAYED	TIME OF SACRIFICE AFTER DOSAGE (HOURS)					
	½	1	2	4	6	24
Brain	46*	51	51	39	34	4
Plasma	388	408	336	283	283	62
Kidney	177	196	146	120	117	27

*Micrograms per gram of tissue or milliliter of plasma.

Sodium salicylate, 232 mg. per kilogram, was administered intraperitoneally to rats. At the indicated intervals 3 animals were sacrificed and the concentration of salicylate in brain, plasma, and kidney determined by the described procedure. The values are the means for the 3 animals.

DISCUSSION

Fluorometric assay, when applicable, offers a number of advantages over other methods of assay. Its sensitivity is much higher than that of spectrophotometry and its specificity is greater since it is based on two spectral requirements, activation and emission.

This procedure involving extraction of salicylic acid from tissues and

subsequent spectrophotofluorometric assay is rapid, simple, and specific and is capable of measuring concentrations which heretofore were not measurable.

SUMMARY

A simple, rapid, and specific method is described for the estimation of salicylic acid in tissues. The salicylic acid is separated from tissues by extraction into ether. It is then returned to borate buffer, and subsequently measured in the spectrophotofluorometer using 310 mμ for activation and 400 mμ for emission.

REFERENCES

1. Brodie, B. B., Udenfriend, S., and Coburn, A. F.: The Determination of Salicylic Acid in Plasma, J. Pharmacol. & Exper. Therap. **80**: 114-117, 1944.
2. Smith, M. J. H., and Talbot, J. M.: The Estimation of Plasma-Salicylate Levels, Brit. J. Exper. Path. **31**: 65-69, 1950.
3. Saltzman, A.: Fluorophotometric Method for the Estimation of Salicylate in Blood, J. Biol. Chem. **174**: 399-404, 1948.
4. Smith, P. K., Gleason, H. L., Stoll, C. G., and Ogorzalek, S.: Studies on the Pharmacology of Salicylates, J. Pharmacol. & Exper. Therap. **87**: 237-255, 1946.
5. Ungar, G., Damgaard, E., and Wong, W. K.: Determination of Salicylate Acid and Related Substances in Serum by Ultraviolet Spectrophotometry, Proc. Soc. Exper. Biol. & Med. **80**: 45-47, 1952.
6. Udenfriend, S., Duggan, D. E., Vasta, B. M., and Brodie, B. B.: A Spectrophotofluorometric Study of Organic Compounds of Pharmacological Interest, J. Pharmacol. & Exper. Therap. **120**: 26-32, 1957.

DETERMINATION OF POLYSACCHARIDE HEXOSES AND HEXOSAMINES IN NORMAL HUMAN SERA

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THE serum polysaccharides are bound to proteins constituting the glycoproteins. The carbohydrate component consists usually of galactose, mannose, glucosamine, galactosamine,[1] sialic acid,[2] and fucose.[3] The glycoproteins have been studied in several clinical conditions with very interesting results.[4, 5, 6]

The carbohydrate moiety of these glycoproteins may be determined quantitatively by several methods with different reagents evaluating the hexoses and the hexosamines. Among the processes for the determination of the hexoses there are those utilizing orcinol,[7, 8] carbazole,[9] diphenylamine,[10, 11] tryptophane,[12, 13] anthrone,[14, 15, 16] and resorcinol-disulfonic acid.[17] Each one of these reagents is used with concentrated sulfuric acid. The hexosamines are determined by the Elson and Morgan reaction[18] or one of its modifications.[19, 20, 21, 22]

In this paper we have studied the methods for determination of the total glycoprotein and the perchloric acid soluble seromucoid[6] hexoses in normal persons, with the anthrone reagent and with resorcinol-disulfonic acid, modified by addition of boric acid to the system. The hexosamines were also determined.

MATERIAL AND METHODS

Venous blood serum was obtained in the fasting state from 30 healthy human beings, the serum being used immediately or kept at −30° C. for future use.

Total glycoprotein and seromucoid hexoses were determined by the method of Graff and associates[15] and also by that of Lunt and Suteliffe,[17] with and without the addition of boric acid:

Reagent A – 0.2 per cent anthrone
Reagent B – 0.2 per cent anthrone + 5 per cent boric acid
Reagent C – 0.5 per cent resorcinol-disulfonic acid
Reagent D – 0.5 per cent resorcinol-disulfonic acid + 5 per cent boric acid.

The reagents were dissolved in concentrated sulfonic acid. The color was developed at a temperature of 80° C. for 30 minutes, followed by spontaneous cooling. Total glycoprotein hexosamines were determined by the method of Shetlar and associates.[20]

RESULTS

The results of the determination of total glycoprotein hexoses are shown in Table I.

The lowest values and smallest coefficients of variation are obtained with the anthrone-boric acid reagent (Table I).

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TABLE I. TOTAL GLYCOPROTEIN HEXOSES OF NORMAL SERA

REAGENT	MEN (15 CASES)		WOMEN (15 CASES)	
	MEAN* AND RANGE	V.C.†	MEAN* AND RANGE	V.C.†
A	126.6 (99.8–154.7)	12.7	135.8 (107.2–158.4)	11.5
B	117.1 (99.3–131.8)	8.5	114.5 (102.0–140.7)	8.2
C	189.9 (158.8–233.6)	12.8	176.0 (145.5–233.6)	14.6
D	168.0 (127.3–217.8)	15.7	154.1 (128.0–209.4)	17.8

*Milligram per cent hexoses expressed as galactose-mannose.
†Coefficinet of variation.

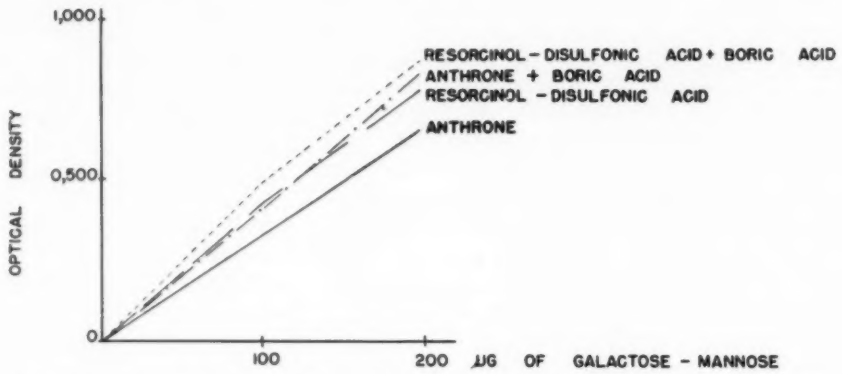

Fig. 1.—Comparison of the optical density curves of the reaction between an equimolar solution of galactose and mannose and several hexose-reagent mixtures.

The values for the seromucoid hexoses are indicated in Table II.

TABLE II. SEROMUCOID HEXOSES OF NORMAL SERUM

REAGENT	MEN (15 CASES)		WOMEN (15 CASES)	
	MEAN AND RANGE	V.C.	MEAN AND RANGE	V.C.
A	11.6 (7.7–15.7)	19.6	8.9 (6.2–13.9)	20.3
B	10.0 (8.3–12.4)	12.0	9.0 (7.5–12.1)	14.4
C	13.6 (9.3–14.2)	20.7	11.5 (9.3–14.2)	15.8
D	12.7 (9.4–15.0)	16.0	11.6 (8.8–14.7)	15.9

For the seromucoid fraction, too, smaller coefficients of variation were obtained with the anthrone-boric acid reagent (Table II).

Table III gives the values for the total glycoprotein hexosamines of the same group of normals.

According to our results, the glycoprotein hexosamines as well as the hexose components were not significantly different in relation to the sex (Tables I, II, and III).

TABLE III. TOTAL GLYCOPROTEIN HEXOSAMINES OF NORMAL SERUM

MEN (15 CASES)		WOMEN (15 CASES)	
MEAN* AND RANGE	V.C.	MEAN AND RANGE	V.C.
75.3 (63.1–86.4)	9.89	72.0 (51.5–80.7)	11.89

*Milligram per cent of hexosamine.

Hexose determinations in the standard equimolecular solution of galactose and mannose by the 4 reagents showed greater optical densities (indicating greater sensitivities) with the boric acid modification at 620 mμ (Fig. 1). Measurements at the wave length of 630 mμ gave identical results.

In a "recovery" experiment where two sera with different known concentrations were mixed the most consistent results were obtained with the anthrone-boric acid reagent (Table IV).

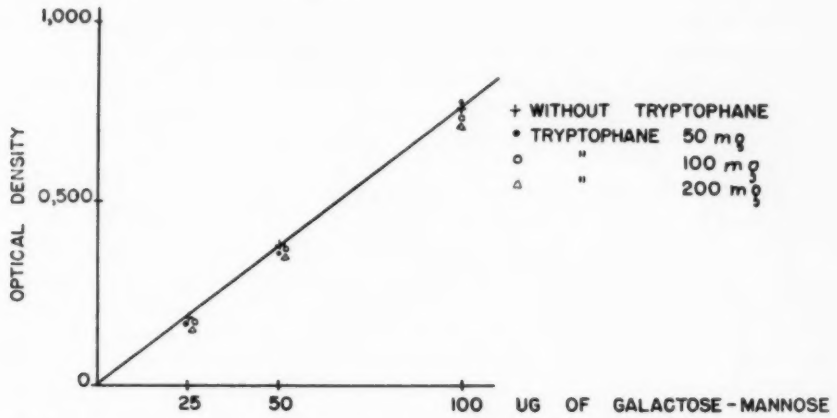

Fig. 2.—Influence of tryptophane in the reaction between the anthrone-boric acid reagent and an equimolar mixture of galactose and mannose.

TABLE IV. "RECOVERY" EXPERIMENTS FOR GLYCOPROTEIN HEXOSES

SERUM A SERUM B SALINE	1.0 ML. 0 4.0	1.0 ML. 0.5 0	1.0 ML. 1.0 0	0.5 ML. 1.0 0
Reagent A	126.3*	100.1	98.7	100.4
Reagent B	90.8	97.8	98.7	99.8
Reagent C	128.5	93.2	89.6	91.1
Reagent D	134.1	92.2	89.3	88.0

*"Recovery" expressed as percentage of theoretical value.

Using the anthrone-boric acid reagent with the galactose-mannose solution, tryptophane[16] is found to have no influence (Fig. 2) whereas Shetlar[23] observed that tryptophane reacted with anthrone thus interfering with the carbohydrate reaction.

Our results for seromucoid and total glycoprotein hexoses and hexosamines agree within fair limits with those reported by others.[7, 9, 12, 16, 19, 20, 22, 24-32]

DISCUSSION

The colorimetric procedures used (anthrone and resorcinol-disulfonic acid, with and without boric acid) are based upon the transformation of the glycoprotein carbohydrate moiety into furfural derivatives.[33] Differences in the carbohydrate behavior have been utilized by some authors[34-37] for analytical purposes, adapting the anthrone method to distinguish between different types of sugars. The color developed by the chromogenic agent is related to the concentration of a number of intermediates which form during the progressive destruction of the sugar, making it impossible to apply this reaction to heterogeneous mixtures obtained by the hydrolysis of serum glycoproteins. Reproducible results may be obtained, however, by standardizing the conditions of the reaction. For that reason we have studied some of the factors which could determine variations in the final result, with special reference to the quality of the reagent and the reaction temperature. In relation to the first factor we consider it unnecessary to prepare the anthrone solution at the moment of use, as recommended by the majority of authors. The reagent can be kept in a well-stoppered bottle protected from light in a deepfreezer for 4 or more weeks. Roe[38] attributes the instability of the reagent to oxidative modifications, with decreasing chromogenic properties, and recommends the use of antioxidants such as 1 per cent thiourea, besides keeping the reagent in the refrigerator.

For color development, a temperature of 80° C. is maintained for 30 minutes followed by spontaneous cooling, differing from the procedure of Mokrasch[34] who also warms for 30 minutes but cools in ice. According to Morris[39] rapid cooling could prevent the end of the reaction causing errors in the determination.

Our results give evidence for the superiority of the anthrone-boric acid reagent over the others, with smaller coefficients of variation, not only for the seromucoid but also for the total glycoprotein hexoses (Tables I and II). Besides, the values obtained with the standard (equimolecular solution of galactose and mannose) demonstrate greater sensitivity of this reagent by the greater optical density increment (Fig. 1). On the other side, the serum analyses indicate that after the addition of boric acid there is a fall in the hexose values.

For checking our results we have mixed 2 sera, with different concentrations of glycoproteins, and analyzed the components of the various mixtures in known proportions, comparing the results obtained with the theoretical values (Table IV).

SUMMARY AND CONCLUSIONS

1. The anthrone reagent is recommended for the determination of serum polysaccharide hexoses in view of its high specificity.

2. The addition of boric acid to the anthrone reagent increases its sensitivity.

3. Values obtained by the use of this modified reagent in the determination of total glycoprotein and seromucoid hexoses in 30 normal individuals are found to be within the limits observed by other authors. The values found were: (a) Total glycoprotein hexoses, 115.8 mg. per cent ± 9.70 (99.3-140.7); (b) seromucoid hexoses, 9.5 mg. per cent ± 1.25 (7.5-12.4).

4. Simultaneous determinations of protein-bound hexosamines by the Elson and Morgan technique gave the following result: 73.6 mg. per cent ± 8.00 (51.5-86.4).

REFERENCES

1. Meyer, K.: Mucoproteins and Mucoids, *in* Cole, W. H., editor: Symposium on Some Conjugated Proteins, New Brunswick, N. J., 1953, Rutgers University Press, Vol. 9, p. 64.
2. Werner, I.: Hexosamine Component of Seromucoid, Acta chem. scandinav. **5**: 1396, 1951.
3. Dische, Z., and Osnos, M.: Mucopolysaccharides of Glycoproteins of Human Serum, Federation Proc. **11**: 202, 1952.
4. Greenspan, F. M.: Survey of Clinical Significance of Serum Mucoprotein Level, Arch. Int. Med. **93**: 863, 1954.
5. Kushner, D. S., Dyniewicz, H., Duvin, A., and Popper, H.: Correlation of Serum Mucoprotein and Gamma Globulin in Various Disease States, J. LAB. & CLIN. MED. **44**: 823, 1954.
6. Winzler, R. J.: Determination of Serum Glycoproteins, *in* Glick, D., editor: Methods of Biochemical Analysis, New York, 1955, Interscience Publishers, Vol. 2, p. 282.
7. Lustig, B., and Langer, A.: Über die Bestimmung und den Gehalt an freiem, Eiweiss- und gebundenem Nicht-eiweisszucker im normalen und pathologischen Serum, Liquor und Harn, Biochem. Ztschr. **242**: 320, 1931.
8. Sörensen, M., and Haugaard, G.: Über die Anwendbarkeit der Orcinreaktion zur Bestimmung der Art und Menge von Kohlenhydratgruppen in Eiweissstoffen, Biochem. Ztschr. **260**: 247, 1933.
9. Seibert, F. B., and Atno, A. J.: Determination of Polysaccharide in Serum, J. Biol. Chem. **163**: 511, 1946.
10. Niazi, S., and State, D.: The Diphenylamine Reaction of Human Serum, Cancer Res. **8**: 653, 1948.
11. Ayala, W., Moore, L. V., and Hess, E. L.: The Purple Color Reaction Given by the Diphenylamine Reagent; With Normal and Rheumatic Fever Sera, J. Clin. Invest. **30**: 781, 1951.
12. Shetlar, M. R., Foster, J. V., and Everett, M. R.: Determination of Serum Polysaccharides by the Tryptophane Reaction, Proc. Soc. Exper. Biol. & Med. **67**: 125, 1948.
13. Badin, J., Jackson, C., and Schubert, M.: Improved Method for Determination of Plasma Polysaccharides With Tryptophane, Proc. Soc. Exper. Biol & Med. **84**: 288, 1953.
14. Seifert, S., Dayton, S., Novic, B., and Muntwyler, E.: The Estimation of Glycogen With the Anthrone Reagent, Arch. Biochem. **25**: 191, 1950.
15. Graff, M. M., Greenspan, E. M., Lehman, I. R., and Holechek, J. J.: Estimation of Non-glucosamine Polysaccharides of the Serum With the Anthrone Reagent, J. LAB. & CLIN. MED. **37**: 736, 1951.
16. Björnesjö, K. B.: Analysis of Protein-Bound Serum Polysaccharides With the Anthrone Reagent, Scandinav. J. Clin. & Lab. Invest. **7**: 417, 1955.
17. Lunt, E., and Sutcliffe, D.: A New Colorimetric Reagent for Carbohydrates, Biochem. J. **55**: 122, 1953.
18. Elson, A. L., and Morgan, W. T. J.: A Colorimetric Method for the Determination of Glucosamine and Chondrosamine, Biochem. J. **27**: 1825, 1933.
19. West, R., Clark, D. H., and Kennedy, E. M.: The Concentration of Glucosamine in Normal and Pathological Sera, J. Clin. Invest. **17**: 173, 1938.
20. Shetlar, M. R., Foster, J. V., Kelly, K. H., and Everett, M. R.: Serum Polysaccharide Level in the Normal State, Proc. Soc. Exper. Biol. & Med. **69**: 507, 1948.
21. Rondle, C. M. J., and Morgan, W. T. J.: The Determination of Glucosamine and Galactosamine, Biochem. J. **61**: 586, 1955.
22. Boas, N. F.: Method for the Determination of Hexosamines in Tissues, J. Biol. Chem. **204**: 553, 1953.
23. Shetlar, M. R.: Use of Anthrone Reaction for Determination of Carbohydrates in the Presence of Serum Proteins, Anal. Chem. **24**: 1844, 1952.
24. Winzler, R. J., Devor, A. W., Mehl, J. W., and Smyth, I. M.: Studies on the Mucoproteins of Human Plasma, J. Clin. Invest. **27**: 609, 1948.
25. Shetlar, M. R., Foster, J. V., Kelly, K. H., Shetlar, C. L., Bryan, R. S., and Everett, M. R.: The Serum Polysaccharide Level in Malignancy and in Other Pathological Conditions, Cancer Res. **9**: 515, 1949.
26. Greenspan, E. M., Tepper, B., Terry, L. L., and Schoenback, E. B.: The Serum Mucoproteins as an Aid in the Differentiation of Neoplastic From Primary Parenchymatous Liver Disease, J. LAB. & CLIN. MED. **39**: 44, 1952.
27. Badin, J., and Glyn, J.: Relationship Between Plasma Mucoproteins and Protein Sugar in Patients With Rheumatoid Arthritis Receiving Cortisone, Proc. Soc. Exper. Biol. & Med. **86**: 150, 1954.

28. Berkman, J., Rifkin, H., and Ross, G.: The Serum Polysaccharides in Diabetic Patients With and Without Degenerative Vascular Disease, J. Clin. Invest. **32**: 415, 1953.
29. Goa, J.: On Protein-Bound Carbohydrates in Human Serum, Scandinav. J. Clin. & Lab. Invest. **7**: suppl. 22, 1955.
30. Stary, Z., Bodur, H., Livie, S. G., and Batiyok, E.: Über die Polysaccharide des Blutserums und die bei der Lungentuberkulose auftretenden Typen der Polysaccharidämie, Klin. Wchnschr. **31**: 399, 1953.
31. Nilsson, I.: Über den Glucosamingehalt des Blutserums bei Gesunden und Pneumoniekranken, Biochem. Ztschr. **291**: 254, 1937.
32. Rosemberg, C., and Schloss, B.: Plasma Hexosamine Levels in Acute Rheumatic Fever, Am. Heart J. **38**: 872, 1949.
33. Wolfrom, M. L., Schuetz, R. D., and Cavalieri, L. F.: Chemical Interactions of Amino Compounds and Sugars. III. The Conversion of D-Glucose to 5-Hydroxymethylfurfuraldehyde, J. Am. Chem. Soc. **70**: 514, 1948.
34. Mokrasch, L. C.: Analysis of Hexose Phosphates and Sugar Mixtures With the Anthrone Reagent, J. Biol. Chem. **208**: 55, 1954.
35. Koehler, L. H.: Differentiation of Carbohydrates by Anthrone Reaction Rate and Color Intensity, Anal. Chem. **24**: 1576, 1952.
36. Johanson, R.: Anthrone in the Estimation of Hexose Sugars, Anal. Chem. **26**: 1331, 1954.
37. Bouting, S. L.: Differential Determination of Glucose and Fructose in Microgram Quantities, Arch. Biochem. **52**: 272, 1954.
38. Roe, J. H.: The Determination of Sugar in Blood and Spinal Fluid With Anthrone, J. Biol. Chem. **212**: 335, 1955.
39. Morris, D. L.: Quantitative Determination of Carbohydrates With Dreywood's Anthrone Reagent, Science **107**: 254, 1948.

A TECHNIQUE FOR THE TITRATION OF "CROSS-REACTING ANTIBODY" IN GROUP O SERUM

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SERUM from group O individuals is characterized by its possession of both anti-A and anti-B isoagglutinins. In addition, some group O sera may contain a third agglutinin, the essential behavior of which may be summarized as follows:

Absorption of a group O serum with a suspension of group A erythrocytes will produce an expected reduction of the anti-A titer of the serum, but this procedure may also result in some degree of reduction of the anti-B titer. Similarly, absorption of the serum with a suspension of group B erythrocytes will result in an expected reduction of anti-B titer but this may also be accompanied by some reduction of the anti-A titer.

The true explanation of this property of group O serum is the subject of controversy. One means of accounting for it would be to postulate the existence of an antibody with conjugate specificities which might be called anti-AB. Wiener[1] is a proponent of an alternative theory which calls for the existence of a separate antigen, designated "C," which is common to group A, group B, and group AB cells. The apparent cross-reactivity of group O serum would then be due to its containing a corresponding antibody, "anti-C." This controversy may be avoided by using the simple descriptive term "cross-reacting antibody" coined by Rosenfield,[2] who has pointed out the significance of this antibody in relation to AB erythroblastosis in the offspring of group O mothers.

The cross-absorption technique, by which the existence of cross-reacting antibody was originally defined, is laborious and does not lend itself readily to quantitative studies. Therefore, we have devised the following technique for its detection and titration.

MATERIALS AND METHODS

Principles of the Method.—Use is made of the phenomenon of mixed agglutination of minor erythrocyte populations.[3] If a suspension of group A red cells is diluted with a suspension of group O cells so that the concentration of group A cells is not greater than

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1 part per 500 parts of O cells, it is possible to sensitize the group A minor population cells with specific isoantibody (such as anti-A) without the occurrence of agglutination. If the suspension is washed to remove all free anti-A, the addition of a further suspension of group A cells to the sensitized population mixture results in the development of characteristic small agglutinates. Each of these agglutinates consists of an original sensitized A cell bound to the added (or "detector") A cells by divalent anti-A molecules.

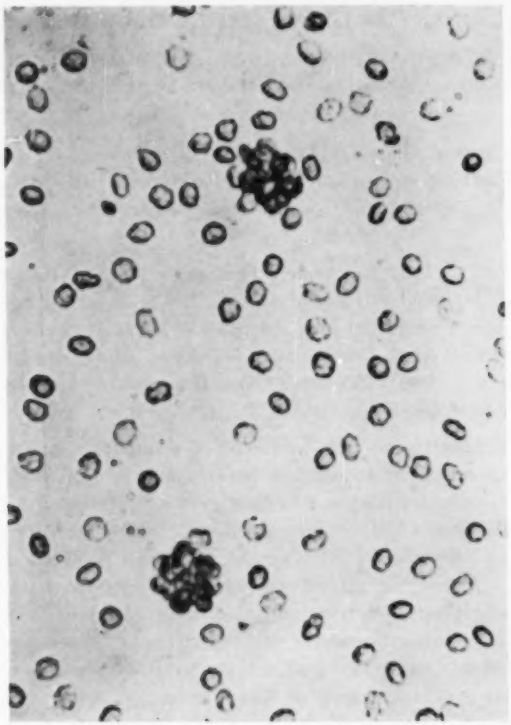

Fig. 1.—The microscopic appearance of mixed agglutinates.

If, instead of using anti-A, a group O serum containing cross-reacting antibody is used to sensitize the group A minor population cells, it is now found that specific mixed agglutinates will be formed not only on the addition of group A detector cells but also on the addition of group B detector cells. It appears that the group O serum contains an antibody which is capable of uniting a group A cell to a group B cell.

This experiment may be repeated in the same form with group B cells substituted for group A cells as the minor population and group A cells substituted for group B cells as the detector suspension. Since group O serum contains anti-A and anti-B in addition to cross-reacting antibody, a group A minor population sensitized with group O serum will always be able to form mixed agglutinates with group A detector cells and a group B minor population will also always be able to form mixed agglutinates with a group B detector suspension.

The test consists of the attempted demonstration of the specific linkage of group A cells to group B cells through the medium of an antibody contained in group O serum. Group O sera which possess the power of linking A and B cells together are regarded as

possessing cross-reacting antibody. The titration of this cross-reacting antibody is performed by determining the greatest dilution of the serum at which this property can be demonstrated.

Technique.—

Cell suspensions: Approximate 5 per cent suspensions are made of group A_1, group B, and group O red cells by pipetting 2.5 ml. of the twice-washed, packed cells of each type into separate 50 ml. volumetric flasks which are then filled to the mark with saline. This provides enough material for a large number of tests. If smaller quantities are required the appropriate adjustments should be made to this volumetric step. These cell suspensions will be used for the preparation of the population mixtures (see below) and also for the detector suspensions.

Population mixture: Two population mixtures are used in the test. In each case the major component is group O. The minor components are, respectively, group A and group B. It is convenient to symbolize such population mixtures as O(A) and O(B) where the letter in parentheses in each case indicates the antigen of the minor population. The O(A) and O(B) mixtures are prepared by pipetting 0.05 ml. of the 5 per cent suspension of group A and group B cells each into two separate 25 ml. volumetric flasks. Both flasks are then filled to the mark with the 5 per cent suspension of group O cells. Complete dispersion of the minor population is ensured by repeated inversion of the flasks for a period of at least 3 minutes.

Serum to be tested: Although no difficulty has yet been experienced through loss of minor population cells by hemolysins in the serum under test it is deemed advisable to inactivate the serum by heating it at 56° C. for 20 minutes. It is also necessary to treat the serum with a chelating agent to suppress the formation of anomalous mixed agglutinates.[4] The tetrasodium salt of ethylenediaminetetraacetic acid (EDTA) is suitable for this purpose and should be added to the serum immediately before the test in a concentration of approximately 1 mg. per milliliter. The effect of EDTA in suppressing anomalous mixed agglutinates is transitory, remaining reliable for only about 24 hours after its addition to the serum. Failure to add EDTA to the serum prior to test will result in the formation of agglutinates which have been shown in a previous communication[4] to consist of a quasispecific binding of the minor and major population cells.

Isotonic saline: The diluent used in preparing serial dilutions of serum is 0.9 per cent sodium chloride solution. Sodium EDTA is added to the saline in a concentration of 1 mg. per milliliter in order to suppress anomalous mixed agglutination. The addition of EDTA to the serum alone does not suffice to suppress anomalous mixed agglutination in the higher serum dilutions of the titration.

Test Procedure.—The serum to be tested is inactivated and treated with EDTA as described above. Six sets of serial twofold dilutions are made using EDTA-saline as diluent. It is advisable to carry the dilutions to the tenth power (1:1,024). To each tube of the first 3 sets of dilutions is added an equal volume of the O(A) population mixture suspension, and to the second 3 sets of dilutions is added an equal volume of the O(B) suspension. The contents of all tubes are mixed by shaking and left at room temperature for 15 to 20 minutes.

After this incubation period the cell suspensions in all tubes are washed 3 times in isotonic saline, care being taken to avoid loss of cells. It is not necessary to use EDTA-saline for this step. Following the last wash the suspensions are restored approximately to their original volume, and equal volumes of group A, group B, and group O cell suspensions are added to each set of sensitized washed cells as shown in Table I. The added cells are thoroughly interspersed in the population mixture by shaking and inversion.

Reading Procedure.—The tubes containing the composite suspensions are centrifuged at 250 g for 1 minute* following which the cell deposit is gently but completely resuspended

*Consult the manufacturer's speed and force table for the centrifuge used or apply the equation: $RCF = 0.00001118 \times N^2 \times R$, where RCF = centrifugal force × g, N = revolutions per minute, R = rotating radius in centimeters at the outer tip of the tube.

TABLE I

DILUTION SET NO.	POPULATION MIXTURE	GROUP OF ADDED CELLS
1	O(A)	A
2	O(A)	B
3	O(A)	O
4	O(B)	B
5	O(B)	A
6	O(B)	O

The distribution of detector cell suspensions among the 6 sets of tubes containing sensitized, washed population mixtures.

by inversion of the tube. This centrifugation and resuspension is carried out 3 times in all. After the final centrifugation the cell deposit should not be disturbed until immediately prior to reading. Reading is carried out by resuspending the cell deposit in sufficient saline to produce a convenient concentration of cells for observation in a hemocytometer chamber (i.e., about 0.2 per cent cell concentration). The diluted suspension is introduced into the hemocytometer chamber and examined under the microscope for the presence of characteristic agglutinates. The agglutinates are uniform spherical masses of cells about 20 μ in diameter occurring with a frequency of about 2 per field. Their appearance is illustrated in Fig. 1.

The presence of typical mixed agglutinates in the suspension indicates a positive result. The absence of agglutinates indicates a negative result. As would be expected, there are generally fewer clumps in the last two tubes showing a positive result in the titration than in the suspensions exposed to more concentrated serum. There is not, however, the gradual diminution of clump frequency with progressive serum dilutions as is seen in conventional agglutinin titrations. The end point of a mixed agglutination titration tends to be more sharply defined than the end point of an agglutinin titration. The series of tubes to which group O detector cells were added constitute negative controls and no agglutinates should be observed in those suspensions. If agglutinates are observed in the negative control tubes it should be taken as an indication that suppression of anomalous mixed agglutination has not been achieved or that the serum under test contains a specific or nonspecific agglutinin which is clumping the major population cells.

RESULTS AND DISCUSSION

In the system described above, the presence of cross-reacting antibody in group O serum is revealed by the formation of mixed agglutinates in those tubes in which a group B detector suspension has been added to an O(A) population mixture or in those tubes in which a group A detector suspension has been added to an O(B) population mixture. It is to be expected that the addition of group A detector cells to an O(A) population mixture will always produce mixed agglutinates because of the almost invariable presence of anti-A in group O serum. For a similar reason those tubes in which group B detector cells are mixed with the O(B) population mixture will be expected to show mixed agglutinates. The mixed agglutination titer of anti-A and anti-B in a group O serum will parallel, generally, the agglutinin titer. The titer of cross-reacting antibody appears to bear only an approximate relationship to the agglutinin titers. Our current experience suggests that cross-reacting antibody is more likely to be found in group O sera with agglutinin titers for anti-A or anti-B over 1:256 and less likely to be found when the agglutinin titer is below this level. Tests of several hundred sera have not yet disclosed an instance of the titer of cross-reacting antibody being in excess of anti-A or anti-B agglutinins.

Most examples of cross-reacting antibody are found to be "symmetrical" in their behavior. That is, the titer is similar for both O(A) and O(B) population mixtures. However, occasional examples of gross "asymmetry" are encountered. In extreme form an asymmetrical cross-reacting antibody may fail to form mixed agglutinates when group B detectors are added to the sensitized O(A) mixture but will do so when group A detectors are added to the O(B) mixture. It is because of the existence of asymmetrical antibody behavior that both O(A) and O(B) suspensions are used in the test. If cross-reacting antibody invariably exhibited a symmetrical behavior it would be necessary only to use one variety of population mixture, for example O(A).

The significance of the existence of cross-reacting antibody in group O serum is not yet clearly defined. Evidence exists[2] that it may be causally related to erythroblastosis in group A or group B offspring of group O mothers. Current studies by us, to be reported elsewhere, suggest that titration of cross-reacting antibody in maternal serum may be useful in the prenatal diagnosis of this type of erythroblastosis. It is tempting to draw a parallel between the hemolytic effect of maternal serum on fetal red cells and the hemolytic effect of the plasma of the "dangerous universal donor" on the red cells of a group A or group B recipient. On this latter point no direct evidence is available, but the technique described above offers a possible means of investigation.

SUMMARY

A description is given of a new technique for the detection and measurement of cross-reacting antibody which may be present in some group O sera. The method depends on the demonstration of direct linkage of group A cells to group B cells by a component of group O serum, thus avoiding laborious cross-absorption or elution procedures.

REFERENCES

1. Wiener, A. S.: The Blood Factor C of the ABO System With Special Reference to the Rare Blood Group C, Ann. Eugenics **18:** 1-8, 1953.
2. Rosenfield, R. E.: AB Hemolytic Disease of the Newborn: Analysis of 1,480 Cord Blood Specimens With Special Reference to the Direct Antiglobulin Test and the Role of the Group O Mother, Blood **10:** 17-28, 1955.
3. Jones, A. R., and Silver, S.: The Detection of Minor Erythrocyte Populations by Mixed Agglutinates, Blood **13:** 763-772, 1958.
4. Jones, A. R., and Kaneb, L.: A New Property of Iso-agglutinins of the ABO Group System, Blood. (In press.)

PROCEEDINGS OF THE CENTRAL SOCIETY FOR CLINICAL RESEARCH

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ABSTRACTS

1. EFFECT OF CHRONOLOGIC AGING, HYPERTENSION, AND ARTERIOSCLEROSIS ON HUMAN ARTERIAL ELASTICITY

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(INTRODUCED BY FRANCIS D. MURPHY, M.D.)

In the past, arterial elasticity studies have not included determination of elastic loss associated with chronologic aging in the normal subject. Consequently, data on the changes in arterial elasticity associated with hypertensive states have been difficult to evaluate. Indeed, the reliability of the "Arterial Elasticity Index" has been questioned because its measurement depends on the assumption that cardiac stroke output is fixed during the test period. It is our purpose to demonstrate the validity of the "Arterial Elasticity Index" and use it in a study of chronologic aging, hypertension, and arteriosclerosis.

The ratio of the change in arterial pulse pressure to the decrease in diastolic pressure resulting from amyl nitrite inhalation forms the "Arterial Elasticity Index." It is apparent that the reliability of this index depends upon a stable heart rate and a constant stroke volume during the critical period of measurement. In our studies stable heart rate was observed directly from arterial pulse tracings. A constant stroke volume was demonstrated by arterial pulse-contour analysis and by ballistocardiography.

In a group of 114 "normal" subjects from 9 to 95 years old, a rectilinear relationship between chronologic age and "Arterial Elasticity Index" was found. In 79 patients with arterial hypertension "Arterial Elasticity Indices" correlated well with chronologic age ($P < 0.001$), but not with the etiology or severity of the hypertension. An occasional hypertensive patient with associated diabetes and/or unusually severe or prolonged hypertension showed a premature loss of arterial elasticity. Twenty-six other patients had conditions frequently associated with widespread arteriosclerosis: 8, intermittent claudication; 2, hypothyroidism; 1, "pulseless disease"; 5, cerebrovascular accident; 4, premature senility; 5, myocardial infarction; 1, calcific aortic stenosis. Twenty-three of these had "Elasticity Indices" above the upper limits of normal (+2 S.D.) for their respective age groups. In thirty-one diabetic patients premature loss of arterial elasticity was demonstrated. Three young and relatively mild diabetics had a remarkable loss of arterial elasticity.

Our study shows that hypertensive disease is not associated with premature arteriosclerosis. The apparent separation of hypertension into "elastic" and "inelastic" types is an illusion produced by the progressive arteriosclerotic changes of "normal" aging or associated disease.

2. PLATELET FUNCTION IN POLYCYTHEMIA

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Because of the lack of uniform opinion regarding the hemostatic defect in polycythemia, we have attempted to assess platelet function in this disorder. Prothrombin consumption and platelet thromboplastin generation studies were performed in untreated, controlled, and spent polycythemics. Isolated platelets of the same patients were assayed for factor 3 activity. In view of the characteristic tendency of polycythemic clots to lose much of the red cell mass, a quantitative measure of red cell "fall-out" from the retracting clot was devised and applied to all patients. This involved, in essence, a determination of hemoglobin on the serum after removal of the clot.

Results.—Prothrombin consumption was normal when platelet counts were high, but defective when platelets were in normal range. This relationship was consistently observed both during a phase of thrombocythemia and again when platelets were in normal range. Isolated platelets were deficient in factor 3 activity, both on the assay and the platelet thromboplastin generation test. This deficiency was not corrected by mechanical disruption of the platelets. Red cell "fall-out" was excessive in polycythemics, and this defect persisted when red cell mass and fibrinogen levels were within normal range.

These data suggest that qualitative defects in the individual platelet may be masked by increase in the total number of platelets. They indicate that the individual platelet in polycythemia is deficient in factor 3 activity. They suggest that the platelet is abnormally fragile, and therefore not enough intact platelets survive the coagulation processes to play a proper role in clot retraction. Preliminary observations on platelet osmotic fragility are consistent with this thesis.

3. THE EFFECT OF ANTIDIURETIC HORMONE ON THIRST REGULATION

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(INTRODUCED BY DONALD S. AMATUZIO, M.D.)

Anderson has documented the existence of a thirst or drinking center in the hypothalamus adjacent to the site of ADH production. Evidence that ADH directly affects thirst is supported by the following experiments: Hydropenic rats with diabetes inspidus (DI) when subsequently allowed water ad lib. drink avidly, most of the water being excreted despite the water deficit. When vasopressin was given simultaneously with ad lib. water, the animals did not drink and continued to lose weight. This suggests that vasopressin suppressed drinking and thereby, paradoxically, aggravated the water deficit. In another experiment DI rats were allowed food and 1 per cent saline only to drink. Fluid exchange increased markedly in all six rats, reaching a maximum of 518 ml. per day. One rat developed massive anasarca which disappeared when the saline was replaced by water. Laragh produced ascites in DI dogs by constricting the thoracic inferior vena cava. However, this is the only known instance where abnormal water retention occurred as a result of spontaneous drinking in the absence of ADH and may represent an example of disturbed thirst regulation as well as ADH production.

The salt-wasting syndrome in association with a mediastinal tumor has been attributed to inappropriate ADH production (W. Schwartz and associates). Such an individual is now under observation. The findings thus far reveal that

he is unable to excrete an acutely administered water load even when given alcohol intravenously. Large doses of 9-alpha-fluoro-hydrocortisone failed to correct the negative sodium balance but did cause hypokalemia. Two possible explanations for the syndrome which appear even more plausible than inappropriate ADH excretion are: (1) disturbance in thirst regulation leading to excessive drinking (the cases described by Schwartz and associates responded most effectively to water restriction), and (2) the secretion by the adrenal of a salt-excreting hormone. The administration of ADH to normal subjects on ad lib. water intake has been shown to produce temporary water retention, hyponatremia, and salt wasting with eventual escape. Such behavior is *not* observed in DI subjects, suggesting that ADH is suppressing thirst appropriately in DI individuals but not in the normal subject.

4. PERSISTENCE OF HOMOLOGOUS TISSUE REACTIONS FOLLOWING CROSS-TRANSFUSIONS OF DESICCATED LEUKOCYTES

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Previous studies in rabbits showed that under specific circumstances it was always possible to eliminate the inflammatory and destructive effects of homologous tissue reactions within musculofascial cross-transplants. This was done by mutual cross-transfusions of fresh leukocytes from whole blood prior to grafting. The modification following cross-transfusions of leukocytes consisted of a fibrocollagenous encapsulation of the cross-transplant accompanied by varying degrees of curious vascular penetration by the host in the conspicuous absence of inflammation. Additional studies revealed that cross-transfusion of leukocytes stored for 24 hours at +10° C. prior to transfusion elicited identical modifications of homologous tissue reactions to those following cross-transfusion of fresh leukocytes.

In view of the capacity of some bacteria and viruses to retain antigenicity following desiccation, the following experiments were designed to show whether or not leukocytes desiccated before cross-transfusion are capable of eliciting a modified host-homograft interaction similar to that observed following cross-transfusions of fresh or untreated stored homologous leukocytes. Leukocytes, concentrated by the Ottesen-Kline method, of paired rabbits were desiccated at +10° C. for 24 hours. Following this the dried cells were hydrated for one hour at room temperature. Four days following the mutual cross-transfusion of treated leukocytes, cross-homografts of musculofascial tissues were implanted. Grafts removed two weeks later showed classical homologous tissue reactions consisting of lymphocytic infiltration and angiitis superimposed upon the network of repair within the grafts.

Therefore, homologous leukocytes, desiccated under these conditions, are incapable of modifying the recipient and thereby eliminating inflammatory features of the host-homograft interaction.

5. AN EARLY DEFECT IN WILSON'S SYNDROME

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In a family with three siblings, the two older boys had classical Wilson's syndrome. The third brother, aged 11 years, presented a unique opportunity for study of the early, asymptomatic form. Clinical evidence of hepatic disease was noted in 27 of 185 blood relatives of this family examined by us.

By history, general physical and neurological examinations, the boy appeared normal. The following laboratory tests were normal: liver function; blood counts, urea, creatinine, and uric acid clearances. Blood ceruloplasmin was 27.5 mg. per cent (normal greater than 23 mg. per cent). Total plasma copper was 137 μg per cent (usually below normal of 80 to 120 μg per cent in Wilson's syndrome). Urinary copper averaged 65 μg per liter (normal 0-50 μg per liter in our laboratory); but excretion was normal on 11 of 14 daily determinations. Chromatography of urinary amino acids revealed normal quantities and patterns, but alpha-amino nitrogen values were 242 and 406 mg. per 24 hours (upper limit of normal 250). The electroencephalogram was borderline normal; the Halstead Index of Biological Intelligence was 0.5 (compatible with moderate impairment of higher brain function).

Liver biopsy indicated normal tissue histologically. However, rubeanic acid stains revealed very large amounts of copper. Renal biopsy demonstrated mild, nonspecific, chronic glomerulonephritis (electron microscopy) and tubular hydropic change, but no stainable copper. Three hundred microcuries of Cu^{64} orally produced normally shaped blood concentration curves but higher than normal levels from 6 to 24 hours. Urinary excretion of copper was twice normal and fecal excretion was 87 per cent of the ingested dose. Tryptophane load by mouth (2 Gm.) produced abnormal amounts of anthranilic acid and kynurenin in the urine.

These data suggest that abnormal deposition of copper in the liver occurs very early in Wilson's syndrome, before ceruloplasmin deficit or histological cirrhosis can be demonstrated. They tend to support the hypothesis that abnormal amino acid metabolism is an initial defect. We suggest that, as part of this defect, metabolites of amino acids may be formed in abnormal quantities, some promoting copper deposition in the liver and later inhibiting ceruloplasmin.

6. PULMONARY AND PERIPHERAL ARTERIOVENOUS SHUNTS IN PATIENTS WITH CLUBBED FINGERS

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(Introduced by Donald W. Seldin, M.D.)

Eleven patients with clubbed fingers were studied. Blood samples for O_2 and CO_2 content, pH and PO_2 were obtained from brachial artery and cephalic vein. These determinations were made initially on room air breathing, after 100 per cent oxygen breathing (20 minutes), and after exposure of the hand to iced water for 1½ minutes. In two patients, the effect of a peripheral vasodilator was studied.

The arterial oxygen saturation on room air was 59 to 95 per cent (average 86.6 per cent). The oxygen tension of arterial blood (P_aO_2) was 26 to 89 mm. Hg. The A-V oxygen difference across the hands was low, averaging 1.3 vol. per cent (range 0.3 to 3.55 vol. per cent); in only two instances was the A-V oxygen difference across the hands over 1.5 vol. per cent (A-V oxygen saturation difference over 5 per cent).

After 100 per cent oxygen breathing, A-V oxygen content difference across the hands averaged 2.12 vol. per cent (range 0.03 to 6.11 vol. per cent). Arterial oxygen tension (P_aO_2) was determined in 6 patients. The alveolar-arterial (A-a) oxygen tension gradient revealed considerable intrapulmonary shunting in 5 patients. If systemic A-V oxygen difference of 4.3 vol. per cent is assumed, these shunts would range between 15.8-33.0 per cent of the cardiac output.

Exposure of the hand to iced water (5° to 8° C.) resulted in an increase in A-V oxygen difference across the hand in 6 of the 7 patients; the average increase was 1.56 vol. per cent (11 per cent saturation). The effect of the vasodilator was insignificant.

These observations demonstrate A-V shunting across the hands in patients with clubbing and suggest that many such patients show a significant intrapulmonary shunt. In one patient (with hydatid cyst of the lung), the disturbed circulation in both lung and periphery returned to normal following removal of the cyst.

One must postulate, therefore, the existence of a reversible mechanism, capable of activating A-V shunts in the digits and associated in some way with similar shunts in the lung.

7. AN EVALUATION OF THE RESPIRATORY RESPONSE TO BREATHING 7.5 PER CENT CO_2 IN AIR AS A TEST OF RESPIRATORY CENTER DEPRESSION

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In an individual the magnitude of ventilatory response to elevation of Pa_{CO_2} is dependent upon: (1) the integrity of the respiratory center; (2) the presence of airway obstruction; and (3) other factors. We have studied the ventilatory response to inhalation of 7.5 per cent CO_2 of patients with lung disease and of 6 normal subjects, with and without airway obstruction. We measured the maximal breathing capacity (MBC) and the minute volume ventilation in response to inhalation of 7.5 per cent CO_2 in air ($\dot{V}_{E_{CO_2}}$) during the third minute in all subjects. In normals expiratory airway obstruction was produced by introducing segments 1 inch in length and 7 mm., 5 mm., 3 mm., and 2 mm. in diameter.

Normal subjects without airway obstruction had a mean MBC of 171 L./min. and a mean $\dot{V}_{E_{CO_2}}$ of 35 L./min. The mean values with the 7 mm. airway were MBC 88 L./min., $\dot{V}_{E_{CO_2}}$ 28 L./min.; with the 5 mm. airway, MBC 50 L./min. $\dot{V}_{E_{CO_2}}$ 20 L./min.; with the 3 mm. airway, MBC 35 L./min., $\dot{V}_{E_{CO_2}}$ 17 L./min.; and with the 2 mm. airway, MBC 13 L./min., $\dot{V}_{E_{CO_2}}$ 7 L./min.

Nine patients with emphysema, normal O_2 saturation and normal Pa_{CO_2} had a mean MBC of 52 L./min. and a mean $\dot{V}_{E_{CO_2}}$ of 24 L./min. Eight patients with emphysema, hypoxemia, and normal Pa_{CO_2} had a mean MBC of 41 L./min. and a mean $\dot{V}_{E_{CO_2}}$ of 25 L./min. Fifteen patients with emphysema, hypoxemia, and elevated Pa_{CO_2} had a mean MBC of 26 L./min. and a mean $\dot{V}_{E_{CO_2}}$ of 12 L./min. We studied 49 other patients with lung disease. In these patients and in those with emphysema, decreased ventilatory response to increased Pa_{CO_2} can be explained on the basis of airway obstruction.

In 11 patients with obesity and in 15 patients with myxedema, all with normal O_2 saturation and Pa_{CO_2}, the mean responses to breathing 7.5 per cent CO_2 were less than would be predicted on the basis of their MBC. Since their Pa_{CO_2} was normal, other factors than respiratory center depression and airway obstruction are implicated.

A diminished ventilatory response to elevation of Pa_{CO_2} cannot be interpreted as respiratory center depression without consideration of airway obstruction.

8. FOLLOW-UP STUDIES UPON INFANTS WITH COARCTATION OF THE AORTA AND PATENT DUCTUS ARTERIOSUS

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It has been our feeling that infants born with coaretation of the aorta and an intra- or extra-cardiac shunt frequently develop congestive failure early in life which is resistant to intensive medical treatment. Surgical correction of the defects is, therefore, indicated as a lifesaving procedure.

This is a report on 10 infants with the above malformations who were operated upon. Eight of these patients survived. Of these patients, 7 were in congestive heart failure, and one had a history of failure. Seven of the 8 had marked systolic hypertension and the blood pressure gradients between the upper and lower extremities varied between 40 to 130 mm. Hg.

Seven of the patients were 4 months of age and under at the time of operation and 1 was 18 months old. Seven patients had a patent ductus arteriosus demonstrated at operation in addition to the coaretation.

The survivors have been followed from 1 to 4 years postoperatively and have been re-evaluated. The following information has been gathered: (1) No hypertension has been demonstrated in any patient. (2) Four patients have normal blood pressure gradients between the upper and lower extremities, and 4 patients have only a 10 to 20 mm. Hg gradient. (3) Four patients show definite evidence of an intracardiac defect (1 patient was shown to have an interventricular septal defect). In the remaining 4 patients there is no clinical evidence of additional cardiac defects.

We feel that this experience reaffirms our conviction that early surgical intervention in infants with coaretation and additional cardiac defects is feasible and lifesaving. There are no indications that the coaretation was re-established in these patients during this period of follow-up.

9. I^{131} CONCENTRATION IN HUMAN FETAL THYROID GLAND FROM NUCLEAR WEAPONS TESTING

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Radioactive iodine, I^{131}, from nuclear weapons testing has been found in the thyroid glands of adult farm animals, fetal sheep, and adult human beings. We have studied the concentration of I^{131} in the human fetal thyroid gland.

Thirty-two human fetal thyroid glands from 28 pregnancies, 8 adult human thyroid glands, 25 adult and fetal hog thyroid glands, 12 reagent blanks, and specimens of snow, tap water and milk were analyzed for I^{131} content between Nov. 12, 1958, and Aug. 1, 1959. I^{131} was obtained from samples by a modification of Barker's method and counted in a windowless gas-flow counter in an anticoincidence umbrella in an old iron brick cave, yielding an uncanceled background count of 1.6 counts per minute. Samples showing activity greater than twice the background count were recounted weekly until the fall in count rate plateaued. Samples of known I^{131} concentration were counted as standards.

Radioactivity was found in thyroid specimens from all sources with a single half-life averaging about 8.4 days, and therefore compatible with I^{131}. The maximum concentration was 265 $\mu\mu$c per gram of fetal thyroid tissue, 29.1 $\mu\mu$c

per gram of hog thyroid tissue, and 22.9 $\mu\mu$c per gram of adult thyroid tissue. The highest activity found on a reagent blank was 7.23 $\mu\mu$c. The median I^{131} concentration values were 5.53 $\mu\mu$c per gram of fetal thyroid tissue, 2.4 $\mu\mu$c per gram of hog thyroid tissue, 5.5 $\mu\mu$c per gram of adult thyroid tissue with a median activity of 1.02 $\mu\mu$c for the reagent blanks. Snow contained 11 $\mu\mu$c per 10 ml.

Correlation of data on fetal pathology, age of fetus, thyroid status of mother during pregnancy, and frequency of nuclear weapons testing, with degree of concentration of I^{131} in thyroid tissue revealed that only the frequency of nuclear weapons testing significantly affected the concentration of I^{131} in fetal thyroid. The calculated maximum total radiation delivered to any fetal thyroid gland was 0.35 rad. Extrapolations from data of Bustad on sheep suggest that it is unlikely that this quantity of radiation would produce hypothyroidism or carcinoma of the thyroid gland.

10. DIASTOLIC MOVEMENTS OF THE PRECORDIUM IN PATIENTS WITH MITRAL STENOSIS AND REGURGITATION; A NEW METHOD TO DETERMINE THE HEMODYNAMICS OF THE MITRAL VALVE

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The present study was carried out in order to determine the degree of stenosis or regurgitation of the mitral valve. Apexcardiogram-ACG (movement of the chest wall overlying the left ventricle) was recorded in 45 patients with mitral valve disease and in 30 normal controls. ACG analysis was made with special attention given to the diastolic filling wave which begins at the base of ACG and ends with the beginning of following systolic wave. When the filling wave rose sharply from its origin to a definite peak and then continued to its termination at the beginning of the following systolic wave, the initial early filling component was designated rapid filling wave (RFW) and the delayed component was designated the slow filling wave (SFW). Angles that the RFW and SFW made with base line were designated rapid filling angle (R) and slow filling angle (S), respectively. Total angle (T) was determined by drawing a line from the base of the ACG to the summit of the following systolic wave and measuring the angle between this line and the base line. In order to compare those apex-cardiograms, with different amplitudes, the ratio of the T angles to the filling angles was calculated.

RFW was absent in 24 cases of proved mitral stenosis. S angle ranged from 10° to 30° (mean 22°) and T/S ratio ranged from 1 to 3. SFW was replaced by RFW in 16 patients following mitral valvulotomy. R angle ranged from 31° to 75° (mean of 55°) and T/R ratio varied from 0.52 to 0.98. Twenty patients with dominant mitral regurgitation presented RFW with R angle ranging from 42° to 75° (mean 59°) and the T/R ratio ranged from 0.51 to 0.98 (mean 0.77). Seven patients with mitral stenosis had Ry/V ratios from pulmonary wedge curves of less than 1.4 (Owen and Wood's criteria) as well as apexcardiograms without rapid filling wave, and S angle ranging from 16° to 32°.

As a means of discretely separating predominant mitral stenosis from predominant mitral regurgitation, this method seems to have as much value and reliability as the more complicated intracardiac approach.

11. ABSENCE OF ERYTHROCYTE GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY IN CERTAIN PERUVIAN INDIANS

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Hereditary deficiencies of erythrocyte glucose-6-phosphate dehydrogenase (EGPD) have been found in a number of peoples, and Motulsky has suggested that a decreased susceptibility to falciparum malaria in deficient individuals might explain the persistence of this genetic defect in a population. The present studies were undertaken to accumulate more data which might bear on this problem. It was also planned to use EGPD deficiency as one of several measurements for the hematologic-anthropologic study of certain Peruvian Indians in an attempt to clarify relationships with other ethnic groups, such as the eastern Polynesians as postulated by Heyerdahl in *Kon-Tiki*. EGPD was measured by the brilliant cresyl blue technique of Motulsky. Each of the three Indian groups measured has a small representation of Spanish ancestry, and some intermarriages with other tribes have taken place. The groups are: (1) Aymara-speaking; mostly descendants of the Lupaca tribe: 34 men (20 to 60 years) and 24 women (13 to 90 years) from the village of Jaillihuaya, altitude about 12,800 ft., near Puno and Lake Titicaca in the southern Andes of Peru; (2) Quechua-speaking; mostly descendants of the Colla tribe: 70 men (21 to 69 years) and 50 women (18 to 72 years) from the village of Paucarcolla, altitude about 13,200 ft., also near Puno; (3) descendants of the Motilones (or Lamistas) tribe: 50 men (17 to 26 years) from the Tarapoto area, northeastern jungle foothills of the Andes, about 2,500 ft. altitude. This tribe was settled around A.D. 1440-1470 by a group of Chancas who escaped from Inca domination near Andahuaylas, about 8,000 ft. altitude in the southern Andes, and migrated to this area.

Malaria, both vivax and falciparum, is common in the Tarapoto area. Vivax has occasionally been contracted around 8,000 ft. but not in the Puno area.

In none of the 238 blood samples was EGPD deficiency noted. In summary, EGPD deficiency was not noted in the highland Indians of southern Peru, where malaria has not been a problem, and apparently did not develop during 500 years after resettlement in a highly endemic area for falciparum malaria.

12. THE EFFECT OF IRON THERAPY ON CHRONICALLY TIRED, NONANEMIC WOMEN: A DOUBLE-BLIND STUDY

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Chronic fatigue is one of the most common complaints encountered in clinical medicine. Often no organic disease can be found to account for the patient's symptoms. It has been claimed repeatedly that iron therapy exerts a beneficial effect on the symptoms of such patients even when the hemoglobin level is within the normal range, but no controlled investigations have been carried out. We have recently demonstrated that in experimental animals iron enzymes may be affected by the iron deficiency state even when the hemoglobin level is normal or near-normal. This finding could lend a logical basis for the occurrence of symptoms in nonanemic iron-deficient patients. The current investigation has been undertaken in order to determine whether the chronic fatigue from which *nonanemic*, otherwise healthy, women frequently suffer may be due, in some cases, to iron deficiency without anemia. Because of the subjective nature of the patients' complaints, greatest care was taken in the design

of the study to eliminate bias. Each woman served as her own control: iron and placebo were administered alternately, the order of presentation being varied randomly. Clinical observations of the patients were carried out without knowledge of whether the patient was receiving iron or placebo or what the patient's plasma iron, iron binding capacity, or state of marrow iron stores was. Decreased marrow iron stores were used as the criterion of iron deficiency. A significant increase in the average hemoglobin values of iron-deficient patients occurred when iron was given; no hemoglobin rise occurred when placebo was given. The average hemoglobin level of patients with normal iron stores was affected by neither iron nor placebo. Of 22 iron-deficient patients, 15 achieved greatest symptomatic improvement with iron, 5 with placebo, and 2 were unimproved. In contrast, of 7 nondeficient women, 5 improved more with placebo and only 2 improved more with iron therapy. The results of these studies suggest that in selected nonanemic patients iron therapy may exert a beneficial effect.

13. THE HYPNOTIC EFFECT OF A NEW PROPANEDIOL DICARBAMATE DERIVATE, W 583 (A DOUBLE BLIND, DOUBLE DOSE STUDY)

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(INTRODUCED BY JOHN H. MOYER, M.D.)

W 583 (2-methyl-2-sec-butyl-1, 3-propanediol dicarbamate) differs from meprobamate by an additional methyl group and has a more pronounced hypnotic activity. It was nontoxic except for respiratory depression in animals given high doses. The hypnotic effect of this drug was investigated in 18 human beings suffering from insomnia and night restlessness. A double blind, double dose technique was used, including 200 and 400 mg. doses and two placebos. The hypnotic effect was evaluated in weekly periods by the onset, depth, and duration of the patient's sleep. A known combination of 50 mg. pentobarbital and 200 mg. W 583 was also given for comparison. The response was graded and statistically analyzed.

WM-3 capsules (400 mg. of W 583) showed the best response, 3.11, and WM-1 was next with 1.35. Wm-3 was significantly superior ($p < 0.001$) to the other 3 unknowns and in the same range ($p = 0.09$) of usefulness as the known combination of agents. Likewise WM-1 (200 mg. capsules) was superior ($p < 0.01$) to WM-2, and WM-2 and -4 were essentially equal (placebo). WM-1 (200 mg. W 583) was mildly hypnotic, but doubling the dose brought out the highly significant hypnotic effect of this compound. Potentiation of pentobarbital by W 583 occurred. A relatively slow onset of action (1 to 6 hours) makes this drug suitable for those who are able to fall asleep but wake up during the night. Side effects were not observed.

14. THE INSULIN-LIKE ACTIVITY OF CERTAIN NITROGENOUS COMPOUNDS

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While studying the insulin-like activity of tissue extracts, by using the glucose uptake of paired hemidiaphragms of the rat, insulin-like activity of the media was traced to ammonium ion. This paper reports studies of insulin-like activity of a number of nitrogenous compounds. In these studies glucose uptake from the media and glycogen content of the diaphragm were measured at the end of the incubation period. Significant increase in the glucose uptake

was produced by the following compounds at a concentration of 10^{-3} M: guanidine, l-alanine, l-isoleucine, and glycine. At a concentration of 10^{-2} M, ammonium, l-leucine, and l-arginine were active.

All of the substances having a strong effect on the glucose uptake also evoked a decrease in the muscle glycogen, in contrast to the effect of insulin which is known to increase glycogen. Several other amino acids not having an effect on the glucose uptake also decreased the glycogen. Guanidine completely reversed the epinephrine (0.1 γ per milliliter) block on glucose uptake. In this respect it resembled the effect of phenoformin on the rat diaphragm. None of the substances tested potentiated the effect of insulin on the glucose uptake, and guanidine reversed the insulin effect on glycogen.

Normal postprandial sera consistently caused an increase in the glycogen content of the diaphragm. This would seem to refute the hypothesis of other workers that the insulin-like activity of human plasma is due to its amino acid content. The possibility that pathological levels of guanidine may predispose to hypoglycemia in the late phases of diabetic renal disease must be considered.

Stimulation of the glucose uptake of the rat diaphragm is thus a property shared by many substances; however, insulin is the only substance tested which increases glucose uptake and glycogen content simultaneously.

15. THE HEMODYNAMICS OF THE VALSALVA MANEUVER IN NORMAL AND PREGNANT SUBJECTS

Richard W. Booth, M.D. (By Invitation), Henry C. Mellette, M.D., Ph.D. (By Invitation), Edward D. Swiss, M.D., Ph.D. (By Invitation), and Joseph M. Ryan, M.D., Columbus, Ohio

Pressure and flow changes induced by forced expiration (40 mm. Hg for 40 seconds) were studied in 21 normal (10 males and 11 females) plus 5 pregnant (seventh month) subjects. Right atrial, arterial and intraoral pressures were measured. Indicator dilution cardiac outputs were obtained 5, 15, and 25 seconds after institution of the Valsalva, at release, plus 4 interspersed controls. Mean circulation time (MCT), central blood volumes (CBV), total peripheral resistances (TPR), and net atrial pressures were calculated.

During the strain, net arterial pressures decreased and cardiac output dropped 30 to 50 per cent from control despite tachycardia. CBV decreased, MCT and TPR increased. At the time of release arterial pressures showed the usual increase over control with a bradycardia. Pregnant females showed a normal pressure response to the Valsalva but the degree of change was less marked compared to normal females. At release the cardiac output of both normal and pregnant females increased significantly over control while males returned only to resting level. MCT usually decreased, TPR returned toward normal, but the CBV remained depressed in 70 per cent of males but only 36 per cent of females.

The following conclusions were reached: (1) Both flow and pressure changes were of greater magnitude in normal females than in males. (2) In this study the form and size of the dye curves and hence resultant flow seemed to be determined between 5 and 15 seconds after indicator injection. (3) Gross systolic pressure failed to show the expected drop during the maneuver in one-third of all subjects, pointing out the difficulty of interpreting the "bedside Valsalva" in congestive failure. (4) As the maneuver is prolonged to 25 seconds the flow in males returns toward normal to a greater extent than in females. This "breakthrough phenomenon" may explain the lack of flow overshoot in males at release and may indicate that 40 mm. intrathoracic pressure is

greater stress to females than males. (5) Apparently reliable indicator dilution curves with exponential slopes and gross recirculation were obtained despite injection done during changing states, e.g., at the time of release.

16. THE IMMUNOELECTROPHORETIC CHARACTERIZATION OF AGAMMAGLOBULINEMIC STATES

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Agammaglobulinemia, in both its congenital and acquired form, has been defined by a variety of morphological, physiological and immunological criteria. An accurate identification of both of these forms requires the application of several of the more incisive methods within each of these three parameters. Utilizing the microimmunoelectrophoretic technique of Scheidegger we have made an analysis of 8 patients with the congenital form and 5 patients with the acquired form of the disease. The technique allows a rough approximation of the amount of 7S gamma globulin present. These levels have agreed well with the values obtained by the standard quantitative precipitin determination and the limiting sensitivity of the method. The two beta-2 globulins, previously reported to be absent or greatly diminished in agammaglobulinemia, have not been demonstrated in patients with the congenital disease, while 3 of the 5 patients with the acquired form have one or the other of these two proteins. Two additional proteins not previously described (mobilities of beta-2 or gamma-1 globulins) have been partially characterized in these patients. These proteins are quite probably genetically determined and their incidence in agammaglobulinemia is very different from that of the normal population.

17. INTERACTIONS BETWEEN PLATELETS AND INFLUENZA VIRUS

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Influenza virus infection as observed by one of us during the 1918 pandemic and confirmed during the recent Asian influenza epidemic is associated with moderate decreases in the platelet count in human beings. Decreases are most marked in the first 24 hours after onset and return to normal levels usually occurs by the fifth day. Decreases were moderate, and purpuric skin lesions were not observed. We have also been able to show a fall in platelet counts in mice inoculated intravenously or intranasally with a strain of influenza virus which is virulent for this species. Motulsky has reported that human platelets are agglutinated by several strains of influenza virus in vitro at 4° C. We have confirmed this finding and in addition have shown that the agglutination occurs at 22° and 37° C.

Suspensions of human platelets were prepared from blood, drawn with siliconed equipment and EDTA as anticoagulant, by differential centrifugation at 4° C. A-Weiss-1943 influenza virus was used. It was suspended in chicken allantoic fluid or partially purified in saline after adsorption onto and elution from chicken RBC.

Mixtures of virus and platelets produced agglutination of the platelets after incubation at 4°, 22°, and 37° C. It appears therefore to be a phenomenon which could occur in warm-blooded animals in vivo. With very high concentrations of virus, there was also apparent destruction of platelets. Thus, the adsorption of virus by platelets differs from the agglutination of red cells by

influenza virus in several ways, particularly in the stability of the bond at 37° C. Agglutination of platelets by virus is accompanied by a decrease in hemagglutination titer and virus infectivity in the supernatant fluid. We will discuss the quantitative aspects of this reaction in terms of platelet and virus concentration and the possible role that this phenomenon may play in the control of influenza viremia. We will also consider possible relationships to platelet function in the clotting mechanism.

We will discuss the effect of influenza virus on platelets from various animal species and its relation to susceptibility to influenza virus infection.

18. A NEW SPECIFIC GENETIC TOOL

J. Marion Bryant, M.D., and Richard C. Bozian, M.D. (By Invitation), New York, N. Y., and Nashville, Tenn.

In previous publications from this laboratory, 4 electrocardiographic phenotypes *(L-L, L-R, R-R,* and *R-L),* identified by the *sequence of intraventricular activation,* have been identified in man. These phenotypes are characterized by *right or left-initial septal* with *right or left-late free wall activation.* The data upon which these observations are based were obtained in Virginia, Tennessee, New York, and Michigan. The distribution of phenotypes, in 1,035 subjects (635 healthy subjects and 400 cardiac patients) of heterogenous racial backgrounds, was not influenced by regional location, age, or sex. However, heart disease of moderate or severe—but not minor—degree definitely altered the distribution.

Analysis of the electrocardiographic pedigrees of 45 families, some of which included 4 generations, with a total population of 302 subjects, suggests the following tentative impressions: (1) The 4 phenotypes represent clear-cut, easily recognizable entities, suitable for evaluating as a hereditary trait. (2) The phenotypes appear in filial generations consistent with *4 theoretical genotypes following the laws of simple Mendelian heredity.* (3) Each genotype behaves in a fashion suggesting the presence of *2 pairs of alleles*—one pair determines the sequence of septal activation and the other pair late free-wall activation. (4) Initial left-septal activation is *dominant* over initial-right septal activation, and late right free-wall activation over that of the left free-wall. The dominant and recessive characteristics of the genotypes can be conveniently represented by the following symbols: *Ll, LR, rR,* and *rl.* (5) Only the homozygous *recessive* genotypes always correspond to the phenotypes.

The potential significance of these preliminary observations, *if confirmed by more extensive investigations,* is obviously relative to their possible bearing on congenital and acquired heart disease, as well as other aspects of clinical medicine and human genetics.

19. DIGITAL PRE- AND POSTCAPILLARY VASOCONSTRICTION AND VASODILATATION RESEMBLING PRE- AND POSTGLOMERULAR ARTERIOLAR FUNCTION IN THE KIDNEY

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It is known that the vascular system of the digits of intact man undergoes continuous spontaneous variations in volume. Among the five spontaneous variations in volume previously quantitatively defined were the alpha and beta deflections. These deflections are controlled by the sympathetic nervous system and are not related to variations in arterial blood pressure, such as the Traube-Hering waves.

By means of rheoplethysmography (RPG) it was found that whenever the alpha deflections were ascending digital inflow was increasing, and when descending digital inflow was decreasing. Also, ascending beta deflections indicate increases in digital volume and descending beta deflections indicate decreases in digital volume. Since descending and ascending alpha deflections are found superimposed upon both ascending and descending beta deflections, a descending alpha deflection superimposed upon an ascending beta deflection indicates a decrease in digital inflow due to net constriction of precapillary vessels at the same time that there is net dilatation of posteapillary vessels. An ascending alpha deflection superimposed upon a descending beta deflection reflects net dilatation of precapillary vessels and greater digital inflow simultaneously with net constriction of posteapillary vessels. Thus, in general, alpha deflections reflect net variations in caliber of precapillary vessels and beta deflections reflect net variations in caliber of posteapillary vessels.

This was further tested by RPG recordings obtained during a single pulse cycle when the alpha deflection was descending and beta deflection ascending. Digital inflow was relatively low and posteapillary collecting vessels rapidly accommodated the inflowing blood. The RPG a short time thereafter, when an alpha deflection was ascending and the beta deflection descending, showed rapid inflow due to net precapillary vasodilatation, but the volume inflow (I_v) curve rapidly became horizontal because of net posteapillary vasoconstriction resulting in relatively low capacity and high tone of collecting vessels.

Thus, digital and pre- and posteapillary vessels behave in a manner similar to afferent and efferent glomerular arterioles which regulate the rate of glomerular capillary filtration. In addition, this type of integration in caliber of the pre- and posteapillary vessels is probably concerned with regulating capillary filtration and reabsorption and flow of interstitial fluid and lymph.

20. PHYSICAL PROPERTIES OF SINGLE ELASTIC FIBERS

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(INTRODUCED BY C. BRUCE TAYLOR, M.D.)

Elastic tissue is thought to be important in the mammalian body because of its physical properties. Measurements of these physical properties have not been entirely satisfactory, however, because elastic tissue always occurs mixed with other tissues. In the past, therefore, it has been separated chemically from the accompanying collagen prior to stress-deformation measurements, or the mixed tissue has been measured as a whole. Both procedures interfere with a true evaluation of the properties of elastic tissue itself. Furthermore, elastic tissue always occurs in networks or meshworks, and one does not know from measurements on these structures how much of the elasticity to ascribe to arrangement of the fibers and how much to the inherent longitudinal elasticity of the fibers themselves.

For these reasons we have dissected free individual elastic fibers from the ligamentum nuchae of cattle. These fibers are 200 to 600 microns in length and average 9 microns in diameter. Under a dissecting microscope one can measure their force-deformation characteristics apart from variables of contaminating tissue and arrangement.

We have measured the lengthening due to force in fresh elastic fibers from the ligamentum nuchae of six oxen and compared this with fiber behavior after exposure to formic acid for 20 hours. We have also compared the elastic behavior of single isolated fibers with fibers as they occur in strips of fresh ligamentum nuchae. Each of these groups behaves reproducibly and shows: (a)

The elastic behavior of individual elastic fibers can be measured up to 130 per cent extension. The force-deformation curves exhibit typical "rubberlike elasticity." (b) This elastic behavior cannot be shown to be altered by exposure to 88 per cent formic acid for 20 hours. (c) Single fibers are somewhat less extensible than fibers arranged in fiber networks (in strips). Hence a positional factor is also invoked to explain the elastic behavior of strips.

Such information is applicable to problems of tissue elasticity involving the human lung, urinary bladder, and blood vessels.

21. THE PARALLEL IN VIVO AND IN VITRO BEHAVIOR OF HUMAN CONNECTIVE TISSUE CELLS

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While the sequence of events leading to initiation and resolution of simple inflammation has been explored histologically, knowledge of the concurrent metabolic events has been more elusive. Information concerning factors controlling the initiation, maintenance, and termination of the inflammatory process is of particular importance in relation to the connective tissue diseases characterized by relentless progression of the inflammatory process.

Histochemical and analytical chemical data suggest that the reparative phase of inflammation is characterized by cellular proliferation, accumulation of mucopolysaccharides, and finally by collagen fiber deposition. In the present experiments, connective tissue cells derived from human synovial tissue were grown in monolayer cultures in a semisynthetic medium. During the growth of such cultures, sequential measurements were made of cell numbers, total cell mass, and total culture protein. Concurrent chemical analyses of the culture medium provided information concerning hexose consumption, lactic acid production, and production of a uronic acid-containing mucopolysaccharide.

Rapid cellular proliferation was closely associated with synthesis of increasing amounts of acid mucopolysaccharide, and in several instances by apparent fiber formation in mature cultures. During the early phase of culture proliferation nearly all of the hexose consumed could be accounted for as lactic acid, suggesting that glycolysis will supply the energy necessary for mucopolysaccharide synthesis.

While the gross similarity of the sequential events in the reparative phase of inflammation and the proliferating cell culture could be coincidental, it appears worthy of exploration as a potential tool for studying the events of the inflammatory process in an isolated form.

22. RE-EVALUATION OF THE PLASMA HEMOGLOBIN CONCENTRATION IN NORMAL HUMAN SUBJECTS

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The authors have recently devised a method for blood collection which greatly diminishes artifactual hemolysis. The benzidine method for hemoglobin measurement has been further modified to yield a tenfold increase in sensitivity, permitting reliable measurements in the range 0.2 to 2.0 mg. of hemoglobin per 100 ml. of plasma. With the aid of the above methods a new range of values for maximum plasma hemoglobin concentration in normal subjects has been defined (mean 0.31 mg. per 100 ml., range 0.16 to 0.58 mg. per 100 ml.).

A number of physiologic variables have been examined in the normal subject to determine their effect upon the plasma hemoglobin concentration. Sleep, ordinary ambulatory activity, alimentary hyperlipemia, and the normal

menstrual cycle were not associated with plasma hemoglobin values outside the above-described normal range. Various physiologic stresses were then studied: circulatory stasis in the forearm (for ten minutes at 25 and 46° C.), induced fever and dehydration, labor and parturition, parenteral administration of Adrenalin and of nicotinic acid, and the brief violent neuromuscular and circulatory activity incident to electroconvulsive shock therapy—all yielded normal values for plasma hemoglobin concentration. The only factor found to alter the plasma hemoglobin level in normal subjects was strenuous exercise. Following ten minutes of violent exercise, laboratory personnel exhibited a two- to three-fold rise in plasma hemoglobin values, which returned to normal levels over the succeeding 40 minutes. Sustained violent exercise among professional basketball players during a league contest resulted in values over 15 times the mean for normal subjects. The possibility that the plasma pigment in exercised subjects may be myoglobin rather than hemoglobin is under consideration.

The stability and the low base-line level of the plasma hemoglobin concentration under normal circumstances have important implications for an understanding of normal erythrokinetics. If the mathematical interpretation of plasma hemoglobin disappearance curves recently proposed by Garby and Noyes is accepted, the present authors' data indicate that less than 10 per cent of the normal daily destruction of senescent erythrocytes occurs within the vascular compartment.

23. VENTRICULAR ANEURYSM FOLLOWING MYOCARDIAL INFARCTIONS: RESULTS OF SURGICAL TREATMENT

Don W. Chapman, M.D., and Denton A. Cooley, M.D. (By Invitation), Houston, Texas

Aneurysm of the left ventricle is one of the most common late cardiac complications of severe myocardial infarction. Although rupture of the aneurysm is uncommon, the effect upon cardiac function may be serious, producing a striking reduction in cardiac output. The paradoxical action of the noncontractile sac during systole dissipates the force of the remaining unaffected myocardium. Cardiac catheterization confirms these observations in revealing a reduced cardiac index and evidence of circulatory stasis in the left atrium reflected by increased pulmonary wedge and pulmonary arterial pressures.

During the past two years nine patients were operated upon for postinfarctional ventricular aneurysms after intervals varying from three months to three years following coronary occlusion. Seven survived operation and were clinically improved. Comparison of cardiac catheterization data before and after operation demonstrated a striking improvement in all patients studied. No essential change in the electrocardiographic tracings was noted postoperatively. Marked reduction of the cardiac size and return toward a normal silhouette were noted in all patients. The possibility of utilizing open surgery in the repair of postinfarctional myocardial aneurysms is suggested for a more definitive form of treatment.

24. BACTERIOLOGIC DIAGNOSIS OF URINARY TRACT INFECTION; RESULTS WITH A QUANTITATIVE METHOD OF URINE CULTURE

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Several reported studies have indicated that some contamination from the anterior urethra occurs in a high percentage of urine specimens for culture whether collected by catheterization or by "clean-voided" techniques. In the

present state of our knowledge the distinction between contamination and actual infection can best be accomplished by quantitative cultures of very carefully obtained specimens. The present study was undertaken to try out a quantitative technique, simple enough to be used in hospital practice for its efficacy in distinguishing between contamination and actual urinary tract infections in catheterized specimens from female patients. There were 130 specimens from 111 patients. The culture method consisted in the spreading of urine over the surface of plates of solid media and counting the colonies after incubation.

It was found that the higher the bacterial count the greater the relative number of patients at that range who by other clinical indices presented a picture of urinary tract infection. This was expected. It was further observed that bacterial counts tended to fall below 100 or greater than 100,000 colonies per milliliter (the limits were not sharp) with relatively few between. This phenomenon has been observed by others. It was concluded that one could not take a single figure as the dividing line between infection and contamination but that below 100 colonies per milliliter the probability of contamination and not actual infection was high, and above 100,000 the probability of actual infection was high. Between these figures one must rely on the clinical picture as a whole. The culture method itself proved to be technically easy and accurate enough for the wide range of bacterial counts obtained.

25. HEPATIC STORAGE OF GLUCOSE DURING GLUCOSE INFUSION IN DOGS WITH PORTACAVAL SHUNTS

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(INTRODUCED BY JACK A. PRITCHARD, M.D.)

The effect of glucose infusions on hepatic glucose balance was determined to examine the participation of the liver in the handling of a carbohydrate load. Dogs with portacaval shunts were used, thereby permitting measurement of hepatic rather than splanchnic glucose changes. Hepatic glucose balance was calculated from measurements of hepatic blood flow and arteriohepatic venous glucose concentration differences. After control determinations, changes in hepatic glucose balance were measured at 10-minute intervals for 90 minutes during a glucose infusion.

Net hepatic glucose output (HGO) decreased promptly in 10 dogs given glucose. Ten minutes after glucose (25 to 235 mg. per minute) was started, HGO had decreased from a mean control of 50.8 to 26.9 mg. per minute. At this time arterial glucose concentration had increased from a mean control of 81.8 to 92.4 mg. per cent. HGO continued to decrease and in 7 dogs an actual net uptake of glucose by the liver was observed. This positive balance was found initially at a mean arterial glucose concentration of 115.6 mg. per cent, an increment of 36.3 mg. per cent above control. Once positive balance was established, it persisted throughout the remainder of the glucose infusion. In 3 dogs, the liver continued to store glucose despite an arterial concentration which, after an intial rise, was decreasing.

The slow intravenous administration of glucagon-free insulin (0.1 unit per kilogram per hour) for 40 to 60 minutes prior to starting an infusion of glucose with insulin resulted in hepatic glucose storage at a mean arterial concentration of 92.9 mg. per cent, an increment of only 16.8 mg. per cent above fasting control values. In one dog initial storage occurred when arterial glucose was 12.8 mg. per cent lower than the control value of 82.5.

These data indicate that the liver responds rapidly to a glucose load first by decreasing its net glucose output and then by actually storing glucose. These changes are apparently mediated by insulin which is secreted in response to a rising blood sugar. Quantitatively, these hepatic effects are significant, the liver accounting for as much of the infused glucose load as peripheral utilization.

26. EFFECT OF DIETARY CHOLESTEROL UPON SERUM LIPID LEVELS IN MAN

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Dietary cholesterol is considered to have little influence on serum lipid levels in man but, conversely, alters greatly the serum lipids of experimental animals. This species difference was studied in 6 healthy human subjects under metabolic ward conditions for 21 weeks. After 5 weeks of a general diet 3 subjects (group A) were given a formula diet with 1.7 to 4.8 Gm. of cholesterol supplied by egg yolk and 3 subjects (group B) were given a cholesterol-free diet. The diets of all periods were balanced as to calories and fat (40 per cent of total calories). The iodine number of the fat fed each group was the same (90). One subject in each group was given only 20 Gm. protein per day. After 74 days the groups were reversed: group A was fed a cholesterol-free diet and group B a high cholesterol diet. The study concluded with a general diet. Cholesterol, phospholipid, and triglyceride determinations were made twice weekly on serum drawn in the fasting state.

For group A the mean serum cholesterol rose 30 mg. per 100 ml. from the general to the high cholesterol diet and then fell 102 mg. on the cholesterol-free diet. For group B the mean serum cholesterol fell 61 mg. per 100 ml. from the general to the cholesterol-free diet and rose 55 mg. when the high cholesterol diet was fed. Serum phospholipid and triglyceride changes paralleled the serum cholesterol changes. Serum cholesterol changes did not correlate with nitrogen balances. The serum cholesterol of the subject given the low protein but high cholesterol diet increased 36 mg. per 100 ml. Two subjects in group A lost weight on the high cholesterol diet while their serum cholesterol increased.

These results indicate that the presence or absence of egg yolk in the diet greatly affects the serum lipid levels. Since the fat content (amount and iodine number) was the same for both the high cholesterol and cholesterol-free diets, the cholesterol content of the egg yolk lipids was apparently the responsible substance.

27. EFFECT OF AGING ON LIPOGENESIS BY THE SKIN

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The rate of formation of long chain fatty acids by abdominal skin of Sprague-Dawley rats of different ages has been studied in vitro by 3-hour incubation of a tissue mince with radiocarbon-labeled acetate. The incorporations expressed in terms of per cent radiocarbon label present in the fatty acids per 100 mg. of skin nitrogen are as follows: 1-month-old rats, 7.71 per cent; 2-month-old rats, 4.25 per cent; 4-month-old rats, 5.03 per cent; 8-month-old rats, 2.46 per cent; 2-year-old rats, 2.29 per cent. The value for the 2-month-old rats is significantly less than that for the 1-month- or 4-month-old rats. The same relative changes are observed if incorporation is based on milligrams of fresh

skin or milligrams of dry skin. The mechanism by which the diminished synthesis is brought about is not clear. It may be related to endocrine changes which occur in the rat with aging.

28. CARDIOVASCULAR SURVEY OF A WEST INDIAN NEGRO POPULATION

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Predisposition of the American Negro to hypertensive vascular disease has been variously interpreted as genetic and environmental; suggested environmental factors are psychosocial status and sodium intake. Complexities of these variables in the United States led to a survey of the cardiovascular status of a relatively unmixed Negro population, living in a uniform, apparently stable, simple and noncompetitive society in St. Kitts, West Indies.

The group consisted of nearly 1,700 laboring villagers aged 20 to 49 years; age and sex distributions of the sample corresponded to those of the island population. Distributions of arterial pressures resembled those of Negroes in urban Bahamas and rural Georgia. They differed from those of white populations in greater "prevalences" of hypertension, particularly among women. Urine analyses indicated that sodium chloride excess was not a determinant of diastolic hypertension. Diastolic hypertension in women occurred as a sequel to toxemia of pregnancy and was also associated with infertility. Major parameters of hypertensive disease were evaluated; the data show practicality of assessment of relative severity of this disease in a field survey.

Serum cholesterol concentrations, measured caloric intakes, and proportion of diet calories as fat, especially animal fat, were lower than in North America. Symptoms and electrocardiographic signs of ischemic heart disease were uncommon. Blood hemoglobin concentrations were lower; serum proteins were higher than North American averages, a difference attributable to high gammaglobulin concentrations.

The data provide base lines for studies in other Negro populations. They confirm the Negro's predisposition to hypertension and suggest that this is primarily genetic. Such a factor may account in part for the low prevalence of ischemic heart disease; however, this is probably also influenced by diet and physical activity.

29. A LONG-TERM FOLLOW-UP OF THE RENAL FUNCTION OF HYPERTENSIVE PATIENTS TREATED BY ADRENALECTOMY

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Approximately 10 years ago the Renal Section was requested to evaluate the renal status of a number of patients who had the following characteristics: (1) progressive grade 3 to 4 hypertension, (2) unresponsiveness to medical therapy at that time, (3) borderline renal function as determined by usual clinical methods (blood urea nitrogen, concentrating ability, phenolsulfonphthalein, and urea clearance). Based on determinations of glomerular filtration rate, effective renal plasma and blood flow, and in certain instances Tm_{PAH}, it was decided that 17 patients had no contraindication to hypertensive therapy

by means of subtotal or total adrenalectomy. Of these 17 individuals, 7 have subsequently died or have been lost to follow-up. The remaining 10 patients have had intermittent evaluation of their renal function, utilizing those tests which determined their preoperative selection, over the past 9½ years. The most recent observations show that there has been a significant reduction in mean arterial blood pressure ($P < .001$) associated with a significant decrease in renal peripheral resistance ($P < .01$). Furthermore 7 of the 10 patients have shown either no progression or an improvement in their renal functional status.

Such results suggest that in selected hypertensive patients subtotal or total adrenalectomy may be of value in preventing further renal damage and in reversing the increased renal vascular resistance frequently associated with hypertension.

30. CONTINUOUS VENA CAVAL INFUSION OF PENICILLIN IN THE TREATMENT OF REMARKABLY RESISTANT SUBACUTE BACTERIAL ENDOCARDITIS CAUSED BY STREPTOCOCCUS FECALIS

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Two decades ago, bacterial endocarditis was an almost uniformly fatal disease. One decade ago it was apparent that most patients with *Streptococcus viridans* infections could be cured with penicillin. Recently, physicians have been plagued with therapeutic problems of resistant organisms buried in the endocardial vegetations. Judged by the number of patients involved, the most important of these has been the *Str. fecalis*.

A 34-year-old white bakery worker underwent uterine dilatation and curettage in a community hospital because of metrorrhagia, and the scrapings were reported as necrotic decidual tissue. Subsequently she experienced persistent malaise and mild fever, developed a right pleural effusion, and showed positive tuberculin skin reaction. Admission examination at a district tuberculosis hospital revealed mitral stenosis and insufficiency, bilateral pleural effusion, anemia, accelerated erythrocyte sedimentation, and nail-bed petechiae. Blood cultures yielded a gram-positive coccus, identified ultimately as *Str. fecalis*. The first course of treatment lasted 46 days and included 378 million units of penicillin intramuscularly and 162 grams of novobiocin orally, followed by prompt clinical and bacteriological relapse. A second therapeutic course spanned 38 days and comprised intermittent intravenous penicillin 1,500 million units, streptomycin 54 Gm., and novobiocin 40 Gm., supplemented by oleandomycin and triacetyloleandomycin. Within 10 days she relapsed again and then was transferred to the City of Memphis Hospitals. We gave her 60 million units of penicillin daily by continuous drip into the inferior vena cava through a polyethylene catheter for 42 days (total 2,520 million units), with oral probenecid, and intramuscular streptomycin-dihydrostreptomycin 60 Gm. and chloramphenicol 88 Gm. in 44 days. Four months after completion of this third course she had manifested no clinical or laboratory evidence of relapse, had gained weight, and was feeling well and working regularly.

Subsequently we consulted with Dr. Lester Goldsmith about another patient who had broken through intensive penicillin-streptomycin therapy. He then treated her as described above, with apparent success, inasmuch as she has not relapsed during seven weeks following cessation of therapy. Continuous intensive intravenous penicillin therapy, supplemented by a second bactericidal drug, appears important in these stubborn endocardial infections.

31. GLYCOLYTIC REACTIONS IN THE HEART DURING AND FOLLOWING ANOXIA

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This study is concerned with the effects of temporary interruption and resumption of coronary flow on the glycolytic pathway in heart muscle. A previous report from this laboratory has demonstrated that concentrations of glycogen and adenosine triphosphate (ATP) diminish progressively in the completely anoxic dog heart. Simultaneously, the concentrations of hexosemonophosphate (HMP), inorganic phosphorus, and lactic acid rise. The present study confirms these observations and shows that the myocardial concentration of fructose 1,6 diphosphate (F-1-6-P) and dihydroxyacetone phosphate (DHAP) also fall during anoxia. The results indicate that phosphofructokinase is a rate-limiting enzyme under these conditions.

If, after 15 minutes of anoxia, the dog heart is perfused with oxygenated blood, rapid resynthesis of glycogen and ATP occurs with a concomitant fall in HMP. Glycogen and ATP usually approach preanoxic levels within two minutes, but fall if the perfusion is continued. This secondary decline can be prevented by the addition of sufficient potassium chloride to maintain cardiac arrest. HMP concentration remains low during reperfusion with oxygenated blood even during periods of rapid glycogenolysis.

Perfusion of an already anoxic heart with a deoxygenated buffer solution hastens glycogenolysis while causing a decline in concentrations of HMP and lactic acid. The latter diffuses into the perfusate; however, HMP does not. Neither F-1-6-P nor DHAP increases during anoxic perfusion. This increase in glycogenolysis may result from the washout of lactic acid, thereby altering a regulatory mechanism similar to that proposed by Cori and Cori to take place during muscle contraction. The conditions of anoxic perfusion also favor phosphofructokinase substrate saturation so that the net rate of the reactions from HMP to lactic acid is increased over that of the nonperfused anoxic heart.

In summary, phosphofructokinase is a rate-limiting enzyme during myocardial anoxia; if perfused with oxygenated blood the heart can rapidly resynthesize glycogen and ATP after 15 minutes of anoxia; perfusion with a deoxygenated buffer solution increases the rate of glycogenolysis and produces a fall in HMP.

32. SERUM SULFATION FACTOR ACTIVITY IN PATIENTS WITH SKELETAL GROWTH IMPAIRMENT

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Sulfation factor is the growth hormone dependent component of normal serum which promotes the incorporation of sulfate-S^{35} into chondroitin sulfate by cartilage from hypophysectomized rats in vitro. Sulfation factor has been assayed in the sera of 33 patients exhibiting severe retardation of skeletal growth.

Twenty-one of our patients presented no evidence of gross hypophyseal lesions or extrahypophyseal conditions which could explain the growth failure. Gowth hormone deficiency was suggested by sulfation factor activities less than 0.3 times the reference normal serum. Four of these subjects subsequently achieved normal growth rates or better following treatment with small doses of

human growth hormone for periods from 2 to 11 months. Review of the clinical and laboratory data often failed to provide evidence of panhypopituitarism which would distinguish some of these patients from "primordial" dwarfs. In the remaining 9 patients higher levels of sulfation factor were found indicating incomplete growth hormone deficiency or other causes of growth failure. One 11-year-old girl with a height age of 6 years and normal sulfation factor activity failed to increase skeletal growth during 22 weeks of treatment with human growth hormone.

The growth failure in 12 other patients was associated with clearly evident extrahypophyseal diseases. Sulfation factor activities greater than 0.3 times normal reference serum were found in the serum of 5 of 7 patients with gonadal agenesis with retarded growth, and in one case each of neurofibromatosis and congenital heart disease. Low levels of sulfation factor activity were present in the serum of an untreated cretin but in one cretin after treatment normal levels were found. The sulfation factor level was 0.28 and 0.39 in two patients following operation on a craniopharyngioma.

Conclusion.—The measurement of sulfation factor activity aids in the recognition of pituitary growth hormone deficiency in syndromes of growth retardation. Preliminary experience indicates that it may be a helpful guide for the selection of patients for long-term treatment with human growth hormone.

33. CARCINOID SYNDROME WITH NORMAL 5-HYDROXYINDOLE-ACETIC ACID EXCRETION

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Determination of urine 5-hydroxyindoleacetic acid (5-HIAA) has been widely used for diagnosis in cases of the carcinoid syndrome; 5-HIAA has almost invariably been increased, and a qualitative adaptation of the test to measure total 5-hydroxyindoles is said to be uniformly positive.

The patient, a 25-year-old unmarried woman, was noted to be anemic, with guaiac positive stools in 1955, but proctoscopy and upper and lower gastrointestinal series were unremarkable. She first noted flushing attacks in the summer of 1956. In September, 1958, a qualitative test for 5-hydroxyindoles was negative. At laparotomy in January, 1959, an ileal carcinoid, 1.5 cm. in diameter, was resected. Liver metastases were present, and flushing attacks were unchanged after operation. Subsequently 56 urine specimens were assayed for 5-HIAA, over 3 months. The range of excretion was 0.5 to 7.9 mg. per day, with a mean of 3.8 mg. per day. N-acetyl 5-hydroxytryptamine and 5-hydroxyindoleaceturic acid, other known serotonin metabolites in the urine of patients with carcinoid syndrome, were not identified by paper chromatography. Similarly, after B-glucuronidase incubation the urine showed no increase in serotonin concentration. Total 5-hydroxyindole excretion in the urine was 9.6 and 3.7 mg. per day on two occasions, thus excluding a tumor producing excessive 5-hydroxytryptophan. Plasma 5-HIAA concentration averaged 0.17 μg per ml. (four determinations), and the blood urea nitrogen was 16, suggesting that renal 5-HIAA excretion was not significantly impaired. The tumor metastases contained 92 μg of serotonin per gram.

Serum serotonin was 0.94 μg per milliliter (mean of eight determinations; normal 0.10 to 0.32 μg per milliliter), and plasma serotonin was 0.04 μg per milliliter (mean of six determinations). A skin test with serotonin was negative, and no increase in urinary 5-HIAA after intravenous reserpine occurred.

although there was a delayed rise in plasma 5-HIAA, and an immediate rise in serum serotonin.

Other pertinent procedures showed an elevated urinary tryptophan excretion, increase in pepsin production, and diminished excretion of urine catechol amines.

Treatment with cyproheptadine, a new serotonin antagonist, and isolated hepatic perfusion with 10 mg. of HN_2 effected no clinical or chemical evidence of remission.

34. URINARY EXCRETORY FUNCTION IN COMBINED ANTERIOR AND POSTERIOR PITUITARY INSUFFICIENCY

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Patients with panhypopituitarism or Addison's disease respond to water loading with minimal diuresis. This has been attributed to greater antidiuretic hormone (ADH) activity, or to primary or secondary removal of 11,17-hydroxycorticosteroids. A study was undertaken in a patient with diabetes insipidus and anterior pituitary insufficiency to evaluate the effects of (1) sodium intake, (2) vasopressin, (3) hydropenia and osmotic (mannitol) diuresis before and after a course of cortisone and hydrocortisone. The following functions were studied: urine flow (V), plasma and urine osmolality (P_{Osm}, U_{Osm}), solute output ($U_{Osm}V$), urinary Na concentration (U_{Na}), and minute output ($U_{Na}V$), osmolal (C_{Osm}), free water (C_{H_2O}) and endogenous creatinine (C_{Cr}) clearances. During high sodium intake U_{Osm} decreased, whereas V, $U_{Osm}V$ and C_{H_2O} greatly increased. These changes were promptly reversed by low sodium intake. The antidiuretic response to vasopressin (Pitressin) was not affected by high or low sodium intake, nor by steroid treatment. Induction of osmotic diuresis following this treatment produced marked increases in V, $U_{Osm}V$, U_{Na}, $U_{Na}V$. C_{Osm} decreased; C_{Cr} did not change.

In conclusion, the polyuria of diabetes insipidus is not dependent on the lack of ADH alone, but also on the renal excretory load. The antidiuretic response to exogenous vasopressin is not affected by 11,17-hydroxycorticosteroids. These hormones in the absence of ADH activity may increase diuresis by causing greater excretory load and/or by decreasing tubular reabsorption of solute-free water.

35. MAGNESIUM METABOLISM IN HYPERTHYROIDISM

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It is known that thyroxine increases the oxidation of glucose without a corresponding increase in high energy phosphate bond formation (uncoupling of oxidative phosphorylation). More recently it has been shown that magnesium deficiency in animals is also associated with uncoupling of oxidative phosphorylation. This study was undertaken to gain further information about the controversy as to whether serum and ultrafiltrable magnesium levels are low in hyperthyroidism as reported by some laboratories or normal as reported by others.

Seven subjects with diffuse toxic goiter were studied before and after successful I^{131} therapy. The mean serum magnesium level was low, 1.57 mEq. per liter, and rose significantly to 1.92 after therapy, and the mean ultrafiltrable magnesium concentration rose from 0.92 to 1.06 mEq. per liter. In addition, a

rise in erythrocyte magnesium concentration from 3.53 to 3.88 mEq. per liter was noted. An elevation of erythrocyte sodium was noted during the toxic state (20.6 mEq. per liter) with a return to normal after the subjects became euthyroid (14.9 mEq. per liter). The erythrocyte potassium was 100.3 mEq. per liter and underwent a significant drop after therapy to 97.4.

Three subjects with toxic nodular goiter were also studied. The serum and ultrafiltrable magnesium levels were not low (2.06 and 1.26 mEq. per liter, respectively) and are actually high normal. Erythrocyte sodium concentration however was elevated similarly to the diffuse hyperplasia group.

Collection of samples anaerobically with ultrafiltration under 5 per cent CO_2 tension did not cause a significant change in the ultrafiltrable magnesium values obtained when compared with paired samples handled without anaerobic precautions.

Conclusions.—(1) Total and ultrafiltrable serum magnesium levels are low in diffuse toxic goiter and these values return to normal with successful therapy. (2) Toxic nodular goiter has normal to elevated serum magnesium concentrations. This finding may aid in explaining some of the conflicting reports in the literature. (3) Both forms of hyperthyroidism have elevated erythrocyte sodium levels.

36. PROLONGED SURVIVAL OF DYSTROPHIC MICE TREATED WITH NORETHANDROLONE

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(INTRODUCED BY A. R. COLWELL, M.D.)

Dystrophic mice, a strain 129 mutant which suffer from a hereditary disease resembling human muscular dystrophy, given 0.5 γ per gram body weight norethrandrolone in corn oil by stomach tube 3 times weekly beginning at 5 weeks of age had a median survival (ST_{50}) of 22 weeks in contrast to control dystrophic mice which had a ST_{50} of 13 weeks. In animals treated with 5.0 γ per gram norethandrolone the ST_{50} was 33 weeks. Force pair fed controls had a ST_{50} of 15 weeks. Animals given 5.0 γ per gram 17α-ethyl-Δ^4-androstenediol-3α,17β-3-propionate intramuscularly weekly had a ST_{50} of 20 weeks.

Norethandrolone does not result in a reversal of loss of muscle function occurring before the start of treatment; it seems only to retard the progress of the disease. Presumably its action is not due to androgenic activity because animals receiving 0.5 γ per gram testosterone propionate intramuscularly three times weekly had a ST_{50} of 15 weeks, not different statistically from the ST_{50} of the control mice. The mechanism of action of norethandrolone is not thought to be related to the increased endogenous synthesis of creatine which follows its administration.

37. METABOLIC EFFECTS OF BLOOD VOLUME REPLACEMENT WITH WHOLE BLOOD AND ERYTHROCYTE FREE SOLUTIONS IN DOGS WITH HEMORRHAGIC SHOCK

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Considerable alteration in the metabolism of glucose occurs during hemorrhagic shock, presumably due to decreased tissue oxidation. The blood concentrations of glucose, pyruvic and lactic acids, and inorganic phosphorus increase and the pH decreases. Reinfusion of the withdrawn blood causes a rise in

blood pressure and a marked improvement in the metabolic abnormalities. The importance of the erythrocyte mass in achieving this beneficial result remained to be determined.

The present study compares, in dogs, the metabolic effect of restoring the blood volume both with and without replacement of the red cell mass. Reduction of the mean arterial blood pressure by hemorrhage to 50 and 30 mm. Hg by the Selkurt-Wiggers technique resulted in a substantial rise in blood glucose, pyruvic and lactic acids, lactate/pyruvate ratio, inorganic phosphorus and a fall in pH. If the blood volume was not replaced, or if saline was substituted for reinfusion of whole blood, the animal died without any improvement in metabolism. However, when the blood volume was reconstituted by either the withdrawn blood or by dextran in saline, the metabolic alterations returned to or near the control values. Improvement was similar with the use of blood or dextran. Dogs whose blood volume was reconstituted with dextran reflected the failure to restore red cell mass, by a marked fall in hematocrit to 11-29 per cent.

The results suggest that sufficient numbers of erythrocytes remain following severe hemorrhage to provide adequate tissue oxidation if an effective circulating blood volume is restored. Replacement of the red cell mass, lost during experimental hypotension, was not required to promote improved glucose metabolism.

38. OBSERVATIONS UPON THE OVERSHOOT-PHENOMENON FOLLOWING POSTURAL HYPOTENSION

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There may be considerable source of error in recording blood pressure following an episode of postural hypotension as the systolic and diastolic values may rise above control levels upon resumption of the supine position. This phenomenon is referred to as "overshoot"; it occurs in normal people and hypertensive patients of various origins. This phenomenon has been investigated in 25 adults, 20 hypertensive and 4 normotensive; 1 had diabetic neuropathy involving the autonomic nervous system. Twenty persons received Guanethidine intravenously (0.5 mg. per kilogram), which appears to produce a substantial fall of standing pressure in continuously treated patients. One patient was treated with oral Ecolid. Four persons received no hypotensive drugs before the tests. Blood pressure was continuously recorded through an indwelling polyethylene catheter in the brachial artery, transduced through a Statham gauge to a Sanborn recorder. The blood pressure response was examined in all persons on a tilt table at 60 degrees. They all exhibited a postural hypotensive response and 15 showed the overshoot phenomenon which, expressed as a percentage of the pretilt control pressures, averaged 21 per cent (5 to 72 per cent) for systolic and 13 per cent (2 to 45 per cent) for diastolic pressure. The overshoot peak appeared at 2¼ minutes (1½ to 4 minutes) following resumption of the supine position; the duration of the whole pressor phase averaged 7 minutes (3½ to 12 minutes). This overshoot has two distinct phases: first, a transient slight elevation of systolic and diastolic pressure, lasting 15 to 20 seconds, occurred immediately after completion of the tilt. This initial response is considered to be due to increased venous return, since it did not occur, or was greatly decreased, when venous pooling was prevented by using an antigravity suit. Second, the main element of this pressor phase, which is prolonged for several minutes, is thought to be caused by

catecholamine release induced by orthostasis. The overshoot phenomenon can be abolished by administration of adrenolytic drugs (Regitine, 5 mg. intravenously) immediately prior to, or during, the tilt procedure. The overshoot phenomenon is believed to have important clinical significance, emphasizing the need of prolonged recumbency before recording pressures, especially in patients receiving drugs likely to cause postural hypotension.

39. ENZYMATIC ACTIVITY OF THE ISOLATED GLOMERULUS IN NORMAL AND NEPHROTIC RATS

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Recent observations have provided evidence that the glomerulus is the site of the earliest renal lesion in aminonucleoside-induced nephrosis, with alterations evident prior to development of proteinuria (Recant and Harkin). Such data adduced by electron microscopy show, in addition, progressive involvement of the glomerulus with duration of the disease. In view of the possible action of the aminonucleoside as an antimetabolite of adenosine, it seemed of interest to investigate the biochemical activity of the glomerulus. This paper is concerned with the definition of the normal enzymatic complement of the glomerulus and the alterations occurring with nephrosis.

Six animals with nephrosis of varying stages of severity (75 to 216 mg. proteinuria per day) were prepared with 6 pair-fed control animals of the same age, sex, and weight. The microchemical methods of Lowry were adapted for study of kidney tissue. Single glomeruli (0.015 to 0.11 μg weight) and tubules were dissected at a magnification of ×90 from rapidly frozen-dried sections of 28 μ thickness and analyses were made of the following enzymes: alkaline phosphatase (AP'tase), lactic dehydrogenase (LDH), malic dehydrogenase (MDH), isocitric dehydrogenase (ICDH), glucose-6-phosphate dehydrogenase (G-6-DH) and hexokinase (HK). Reference base lines were dry weight and protein. Activities were expressed as M substrate utilized per kilogram per hour.

In normal glomeruli the highest activities found were MDH (32 ± 1.7) and LDH (26.6 ± 3.0). AP'tase (3.07 ± 0.33) and ICDH (4.92 ± 0.49) were next, and HK (0.53 ± 0.03) and G-6-DH (1.19 ± 0.098) enzymes were least active. Glomerular activities differed significantly from tubular enzymes. Nephrotic glomeruli showed a marked decrease in AP'tase activity ($P = 0.01$), while G-6-DH activity showed an equivalent rise ($P < 0.005$). LDH ($P = 0.05$) and MDH ($P = 0.01$) activity were also increased. ICDH and HK showed insignificant changes. In order to determine whether the glomerular enzyme changes in the nephrotic were part of a general pattern of reaction or were more specific, spot enzymatic assays of pancreas and adrenal were done.

The observations made suggest the following: (1) the normal glomerulus may have relatively active oxidative metabolism, while its glycolytic components may be somewhat less important, and (2) with the development of nephrosis, glomerular enzymatic changes occur which appear to follow a specific pattern with progression of the disease.

40. INHIBITION OF GLUCONEOGENIC ACTION OF CORTISOL IN VITAMIN B_6-DEFICIENT RATS

ALBERT B. EISENSTEIN, M.D., ST. LOUIS, MO.

Recent reports suggest that gluconeogenic action of cortisol may depend upon increased activity of liver glutamic pyruvic transaminase (LGPT) enzyme

system which results from administration of the hormone. If this hypothesis is correct, glucocorticoid activity of cortisol should be less in animals depleted of vitamin B_6 than in normal animals since pyridoxal phosphate is the cofactor for the enzyme. The experiment described here was carried out to compare the effect of cortisol on liver glycogen deposition in vitamin B_6-deficient rats with that in intact animals.

Young male Sprague-Dawley rats were fed a pyridoxine-deficient diet for 52 days. Control animals were pair fed the same diet to which pyridoxine was added. During the last week, half the animals of each group were given 5 mg. of cortisol subcutaneously daily while those remaining were injected with diluent. Food was withheld on the final day, and 10 hours before animals were sacrificed an additional injection of cortisol or diluent was given. Duplicate samples of liver were taken for glycogen determination and the remainder homogenized for measurement of LGPT activity and protein content.

Cortisol administration to control rats resulted in a marked increase in hepatic glycogen content. When the hormone was given to vitamin B_6-deficient rats, however, the increase in liver glycogen was considerably smaller and significantly less than that which occurred in controls ($P < 0.001$). Administration of cortisol to control rats also induced a considerable elevation of LGPT activity but in vitamin B_6-deficient rats the rise in enzyme activity following cortisol was small and significantly less than that which was observed in controls ($P < 0.001$).

Previously, gluconeogenic action of adrenal steroids has been attributed entirely to their chemical structure. Results of this study show, however, that molecular configuration is not the only factor which determines glucocorticoid activity since in vitamin B_6-deficient rats liver glycogen deposition following cortisol was considerably less than that which occurred in normal rats. Thus, it is evident that gluconeogenic activity of adrenal hormones depends not only on their chemical structure but upon the nutritional state of the recipient animal as well.

41. THE INFLUENCE OF HEPATIC AND THYROID FUNCTION ON RESPONSE TO INSULIN: A NEW METHOD FOR MEASURING INSULIN SENSITIVITY

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(INTRODUCED BY ROBERT J. GLASER, M.D.)

The current method for measuring insulin sensitivity or tolerance is based on the per cent fall of the fasting blood sugar following an intravenous dose of regular insulin (0.1 unit per kilogram body weight). We have developed a new test of insulin responsiveness which is a kinetic one, avoids hypoglycemia and employs a much smaller dose of insulin. The new method is based on our finding that the blood glucose disappearance rate constant ($K_1 = 0.0366$) during a constant intravenous glucose infusion is not significantly different from the glucose disappearance rate constant ($K_2 = 0.0391$) immediately following the cessation of the glucose infusion. Thus, if insulin is administered at the end of the glucose infusion, the difference between the two rate constants ($K_2 - K_1$) represents the influence of insulin on glucose disappearance. Ten per cent glucose is administered intravenously (3.5 ml. per minute) with an infusion pump for 60 minutes and immediately thereafter 3 units of regular insulin are given intravenously. Capillary blood for glucose analysis is obtained at 5- or 10-minute intervals during the glucose infusion and following the

insulin. The rate constants are calculated according to a new method of data analysis (slope analysis).

Studies were carried out in 44 "normal" patients, 30 patients with parenchymatous liver disease, 9 thyrotoxic patients, and 6 patients with myxedema. The response of the patients with liver disease was significantly different from that of the normals: The normals showed a significant increase in the difference ($K_2 - K_1$) (mean: 58 per cent), whereas the liver cases showed no significant increase (mean: 12 per cent). The response of the thyrotoxic patients to insulin was greater than of the normals (mean increase in K: 91 per cent). In contrast, the patients with myxedema showed little or no response to insulin.

The new test has certain theoretical and practical advantages over the currently used insulin tolerance test. The initial findings demonstrate in a dynamic way the marked influence of hepatic and thyroid functions on the blood sugar response to insulin. The implications of the findings and the applicability of the technique to various disease states as well as action of drugs will be discussed.

42. THE NATURAL HISTORY OF INFECTIOUS MONONUCLEOSIS: AN EPIDEMIOLOGIC HYPOTHESIS

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A concept of the natural history of infectious mononucleosis has emerged from an intensive study of this disease over the past five years in over 250 students at the University of Wisconsin. The hypothesis is based on the following observations: (1) the disease is of low apparent contagiousness in this young adult population; (2) the hematologic and serologic changes are usually well developed at the time of hospital admission or within five days thereafter, indicating that it is later than we think in the stage of illness when clinical symptoms appear; (3) the incubation period from exposure to the onset of systemic symptoms is of the order of one month; (4) the heterophile-positive and heterophile-negative illnesses represent the same disease: there are no important qualitative differences between them but quantitatively the heterophile positive cases are more severe in a number of ways.

A theory of pathogenesis will be presented which explains many of the unique epidemiologic and clinical aspects of infectious mononucleosis. The main element of this theory is that there is an abortive, primary phase characteristic of childhood and a later, secondary glandular phase characteristic of the young adult. The former has a short incubation period, frequently an uncharacteristic clinical picture that does not permit diagnosis, and in childhood a high infectiousness leading to widespread immunity; the latter may have a long, apparent incubation period because the primary phase may be absent, is of low apparent contagiousness because there are more immune individuals from childhood infection than susceptibles, and is characterized clinically by systemic lymphatic involvement, hematologic changes, and, if severe enough, by the appearance of heterophile antibody.

43. BILE ACID EXCRETION IN MAN FOLLOWING THE ADMINISTRATION OF THYROID HORMONES

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(INTRODUCED BY WILLIAM H. BOND, M.D.)

The bile acids have been shown to be the principal excretory products of cholesterol, and the chief acids found in man are cholic, chenodeoxycholic, and deoxycholic. In view of the reciprocal relationship between serum cholesterol

level and the degree of thyroid activity, studies were undertaken measuring the effect of 3:5:3′ triiodothyronine upon the composition of biliary bile acid. Studies were made over a 24-hour period. Eight medicated patients were studied by intubation and bile was obtained by duodenal drainage. Dosage of 3:5:3′ triiodothyronine varied from 200 to 500 mcg. and was administered either orally or intravenously in a single dose. Hydrolyzed bile was separated on Celite columns, and the individual bile acids were subsequently measured colorimetrically and spectrophotometrically.

All patients studied showed a consistent fall in the ratio of chenodeoxycholic to cholic acid. The maximal fall was noted two hours after administration of the drug. This appears to be one of the earliest demonstrable effects of thyroid hormone. Values had returned to or exceeded control levels at the end of 24 hours. No consistent or significant effect on deoxycholic acid excretion was noted. Four unmedicated controls were similarly studied, and no consistent or significant effect on the ratio of chenodeoxycholic to cholic acid was noted. Five medicated patients with postoperative choledochostomy drainage showed no consistent or significant effect in the ratio of chenodeoxycholic to cholic acid such as was seen in the intact, intubated patients.

This consistent pattern of bile acid excretion in the intact patients medicated with 3:5:3′ triiodothyronine is different from that reported in the bile fistula rat given desiccated thyroid. To elucidate this problem further, studies employing 3:5:3′ triiodothyropropionic acid are in progress and will be reported.

Bile acids in the bile are excreted as conjugates of glycine or taurine. Hyperthyroidism, liver disease, and choledochostomy drainage all may modify the conjugation processes and may underlie differences in excretion between intact and choledochostomy patients. Further studies in this area are in progress and will be reported.

44. AN INTRAVENOUS TOLBUTAMIDE TEST AS AN ADJUNCT IN THE DIAGNOSIS OF FUNCTIONING PANCREATIC ISLET CELL ADENOMAS

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The effect of intravenous sodium tolbutamide (1 Gm. or 30 mg. per kilogram) on blood sugar levels was studied in patients with (1) proved insulomas, (2) suspected insulomas, (3) severe adrenal insufficiency (off glucocorticoids, on desoxycorticosterone), (4) cirrhosis, and (5) in healthy subjects.

Five tests in four patients with insulomas showed a fall in blood sugar to levels below 26 (mean 19) mg. per 100 ml., a 63 (50 to 79) per cent decrease from fasting levels. Ninety to 180 minutes after tolbutamide administration, blood levels were still below 33 mg. per 100 ml., a depression of 46 (34 to 60) per cent.

In 9 healthy subjects the lowest blood sugar level was 31 (mean 45) mg. per 100 ml. The maximum decrease was 43 (21 to 62) per cent. At 90 to 120 minutes levels were above 63 mg. per 100 ml., a decrease of 9 (0 to 22) per cent. Insulomas were strongly suspected in two patients but finally ruled out. The lowest blood sugar after tolbutamide was 43 mg. per 100 ml. (46 per cent fall). At 90 to 180 minutes levels were above 62 mg. per 100 ml. (depression of 0 and 9 per cent). Seven tests in 5 patients with adrenal insufficiency disclosed decreases to a mean of 37 (lowest 27) mg. per 100 ml., a depression of 49 (37 to 63) per cent. However, at 90 to 180 minutes all levels were above 54 mg. per 100 ml., a mean depression of 11 (0 to 22) per cent. In cirrhotics

the maximum fall was less marked while later decreases were in the same range as in adrenal insufficiency.

The most important finding in this study is persistence of the tolbutamide-induced hypoglycemia for 3 hours in the insuloma patients. This persistence of hypoglycemia is of much greater significance than the degree of hypoglycemia induced. This phenomenon may serve as a valuable adjunct in the diagnosis of insulomas. The degree and duration of the hypoglycemia suggest release of excessive amounts of insulin from these adenomas.

45. EFFECTS OF HUMAN AND BOVINE GROWTH HORMONE IN TWO HYPOPHYSECTOMIZED DIABETIC PATIENTS

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Recent studies have emphasized the species specificity of the biologic effects of pituitary growth hormone. Lipid-mobilizing and ketogenic effects in the human being have been established, but it is not clear whether these are primary properties or are secondary to some modification of carbohydrate metabolism.

The action of growth hormone (G. H.) on carbohydrate metabolism and blood lipids was investigated in two hypophysectomized diabetic patients. The first subject, who required insulin, was given bovine G. H. (Somar-A, Armour) intramuscularly in a dose of 50 to 200 mg. per day for 7 days. There was a pronounced increase of blood sugar and urine glucose levels. Blood ketones, nonesterified fatty acids (NEFA), and total lipids were significantly elevated during this period. In the second subject, who required no insulin, human G. H. (Merck) administered intramuscularly in a dose of 10 mg. a day for 4 days resulted in a similar effect on glucose values and NEFA; in addition, nitrogen retention was observed.

Acute experiments with intravenous administration of human G. H. in doses of 5 mg. in the fasting state were carried out to determine the time relationship of the effect on CHO metabolism and lipids. A fourfold increase of the NEFA level and a tenfold elevation of the blood ketones were observed with a peak about 5 hours after onset of the intravenous infusion. The blood sugar remained practically unchanged during the 6-hour period of the experiment. However, a diabetogenic effect was observed after administration of food and persisted for 24 hours. Administration of glucose, glucose + insulin, and insulin alone with G. H. did not suppress the lipid-mobilizing action of the G. H. The insulin action on the blood sugar was markedly inhibited. It appears that the effects of bovine and human G. H. in these subjects were very similar. The rapidity of the lipid-mobilizing and ketogenic effect suggests that this might be a primary effect of G. H. independent from changes in CHO metabolism.

46. OBJECTIVE CHARACTERIZATION OF THE TWO TYPES OF DIABETES MELLITUS

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(Introduced by Alvin Schultz, M.D.)

Although there is general agreement that two clinical types of diabetes mellitus can be distinguished, the insulin-dependent or labile and the insulin-independent or stable, it is often not possible to classify some patients on clinical grounds alone. Vascular complications of diabetes may depend not

only on treatment but also on the inherent character of the disease. It is important, therefore, to establish efficient and objective methods to characterize the type of diabetes in the individual patient. Forty-two adult patients with long-standing diabetes were hospitalized and studied with regard to ketone bodies and nonesterified fatty acids (NEFA) in the blood after insulin withdrawal, sensitivity to insulin as defined by Himsworth, and acute blood sugar response to tolbutamide. Blood ketones showed significant elevation in one group of patients ("insulin-dependent") within 24 hours, while in the second group ("insulin-independent") practically normal levels were observed, even after several days without insulin. Four hours after oral administration of 3 Gm. tolbutamide, blood sugars in the insulin-dependent group either were elevated or remained unchanged, while almost all insulin-independent cases showed a decrease in blood sugar. There was also a marked difference in the behavior of NEFA between the two types of diabetes: on insulin withdrawal the NEFA rise paralleled the ketone bodies in the insulin-dependent group.

It is concluded that it is indeed possible to separate clearly two main groups in diabetes. The most reliable procedure for this purpose is the determination of ketone bodies or NEFA on insulin withdrawal. However, the good correlation with the tolbutamide response test makes it possible to use this latter, more convenient procedure for classifying patients on a larger scale. The insulin sensitivity test of Himsworth, though agreeing in general with the other tests, was less precise and much more laborious.

47. A STUDY OF THE KINETICS OF ERYTHROPOIESIS

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Some aspects of the kinetics of erythropoiesis have been studied in order to examine the action of the erythropoietic stimulating factor, erythropoietin. The transfusion-induced polycythemic mouse was chosen because endogenous erythropoiesis is eliminated without harmful effects to the animal. A purified preparation of erythropoietin, a glycoprotein obtained from plasma of sheep rendered anemic by administration of phenylhydrazine, was used to stimulate erythropoiesis.

The amounts of erythropoietin required for minimal and maximal response, as measured by reticulocytosis, were established. Erythropoietin potency was expressed in units of activity as defined by Goldwasser and White (Fed. Proc. 18: 236, 1959). Six units of erythropoietin, in a single subcutaneous injection, produces a maximal reticulocytosis of 1.3 per cent on the third day. Decreasing responses were obtained with decreasing amounts; as little as 0.75 unit was followed by 0.26 per cent reticulocytes. The time and duration of peak response were independent of the dose.

Following a single maximum stimulating dose, erythrocyte precursors were counted in splenic red pulp of groups of animals sacrificed daily . Proerythroblasts increased from 0.2 per cent of nucleated cells to 0.9 per cent in 24 hours. Normoblasts increased from 0.0 to 9.6 per cent in 72 hours. Also of interest was the observation that more erythropoiesis occurred subcapsularly than centrally.

In order to examine the effect of erythropoietin on proerythroblasts and normoblasts, a second dose was administered to one group of mice at 24 hours when only proerythroblasts were present, and to another group at 48 hours when normoblasts were in abundance and increasing rapidly. In neither instance did reticulocytes appear earlier than they did following a single dose,

nor was the magnitude of reticulocytosis at 72 hours enhanced. From these data we conclude erythropoietin acts primarily by stimulating differentiation of primitive reticulum, and no effect on differentiated erythrocyte precursors (proerythroblasts or normoblasts) could be detected.

48. STUDIES IN TUMOR AUTOIMMUNITY

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Physicians have observed in the natural history of cancer spontaneous remission, postsurgical regression of remaining lesions, regression of lesions distant from irradiated tumor masses, and sudden renewed growth of metastatic nodules many years following the removal of parent tumors. These observations implicate host resistance as a factor in the course of the disease. An animal may develop immunity to certain transplantable cancers, preventing tumor growth following a secondary implantation. Human studies in this area are relatively few.

Fourteen volunteer male patients having terminal cancer were studied. The work on the first 5 patients was a pilot study with deep x-ray therapy. The remaining 9 patients received intramuscular injections of their tumor antigen in Freund's adjuvant. Blood was withdrawn, weekly, before and after radiotherapy or the intramuscular injections of the antigen. The serum antibody titers were measured with the tannic acid treated sheep erythrocyte agglutination test.

Three of the five patients having deep x-ray therapy showed a significant antibody titer rise, suggesting a need for further studies with a more direct approach. It was noted that metastatic nodules distant from the irradiated mass regressed with the rise in antibody titer.

An inflammatory reaction characterized by erythema, swelling, edema, heat, and tenderness at the tumor site was noted 15 to 25 days following injection of the antigen. At the peak antibody titer, 500 ml. of blood was withdrawn and the proteins were fractionated by Cohn method 10. These fractions were utilized to study cytotoxicity on subcutaneous tumor nodules. Sixty-three per cent of the injected subcutaneous nodules became nonpalpable within a period of 10 days. Five per cent grew and became measurable again before the patient expired. Reintroduction of the antibody protein caused the nodules to become nonpalpable. These same Cohn fractions had no effect on the patient's normal tissue.

These data indicate that a humoral cytotoxic antibody can be elicited by an injection of autologous tumor homogenized in Freund's adjuvant. Detailed data on this study will be presented.

49. CHANGING PLASMA POTASSIUM AND THE ELECTROCARDIOGRAM IN DOGS INTOXICATED WITH DIGITOXIN

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In one phase of a study of the relation of cardiac arrhythmias to plasma potassium level the effect of administration of glucose and insulin on the electrocardiogram in digitoxin-intoxicated dogs was evaluated, after the plasma level of this cation had been raised.

Ten mongrel dogs weighing 8 to 12 kilograms were given 0.5 mg. of digitoxin intramuscularly per kilogram of body weight. On the following day the animals were anesthetized with 30 mg. sodium phenobarbital per kilogram. An oscilloscope was used to monitor the electrocardiogram throughout the experiment, and whenever any change was observed, a permanent record was obtained. Isotonic solution of buffered potassium phosphate in distilled water was infused uniformly, using a flow meter, until second degree A-V block was reached. The rate of infusion was constant for any given animal but varied in different animals from 0.6 to 1.37 mEq. per minute (average, 0.99 mEq. per minute). When A-V block appeared, 50 Gm. of glucose and 30 units of regular insulin were given intravenously, and within 30 to 60 minutes after the glucose was injected, potassium was reinfused to A-V block.

In four animals in which it was studied, the urinary excretion of potassium after glucose and insulin infusion was decreased. Control plasma potassium averaged 3.88 mEq. per liter (3.0 to 4.4). An average of 13.39 mEq. (9.0 to 24.0) of potassium was infused and plasma level at the end averaged 7.52 mEq. per liter (6.6 to 8.0). Thirty to 60 minutes after the glucose and insulin had been absorbed and immediately before the second injection of potassium, the plasma potassium averaged 2.82 mEq. per liter (2.0 to 4.5). Infusion of an average of 36.7 mEq. (20.0 to 71.0) of potassium resulted in A-V block and raised the plasma level to an average 7.24 mEq. per liter (6.8 to 8.1). Ventricular arrhythmias were not observed in any of our animals.

The almost identical plasma level of potassium at the end of the two infusions of potassium, even though 2.7 times as much potassium was needed after glucose and insulin to reach the same end point (A-V block), indicates that under the conditions of these experiments the electrocardiographic changes were dependent primarily on levels of extracellular potassium, rather than the intra- to extracellular ratio of this cation.

50. THE INTERRELATIONSHIPS OF GLOMERULAR FILTRATION RATE, TUBULAR REABSORPTION OF PHOSPHATE, AND RENAL PLASMA FLOW IN VARYING DEGREES OF RENAL FAILURE

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To clarify further the physiologic changes accompanying renal degeneration, 25 hypertensive patients with varying degrees of renal failure (GFR range 10 to 130 ml. per minute) were subjected to discrete renal functional testing by standard techniques and the contemporaneous determination of the tubular reabsorption of phosphate (TRP). While attempting to correlate the TRP with renal plasma flow (RPF) (C_{PAH}) certain discrepancies were observed. This necessitated the determination of TRP with and without the contemporaneous determination of RPF (C_{PAH}). Also, RPF (C_{PAH}) was determined with and without the contemporaneous determination of TRP.

The investigation to determine the correlation of glomerular filtration rate (GFR) and TRP under conditions of high phosphate loading (plasma phosphate between 15 and 25 mg. per cent) indicated that TRP ranges from 4 to 5 mg. per minute when the GFR is greater than 60 ml. per minute. However, when the GFR is less than 30 ml. per minute, the TRP proportionally declines to values varying from 0.3 to 2.7 mg. per minute.

TRP was reduced (average 0.9 mg. per minute) in the presence of the low concentration solutions of PAH used to measure RPF. Additional proof that PAH suppressed TRP was afforded by the removal of PAH and the rede-

termination of TRP which then increased (average 3.6 mg. per minute). It was further noted that C_{PAH} was enhanced (from 320 to 416 ml. per minute) by the presence of high plasma phosphate concentrations. The glomerular filtration of phosphate remained the same in both phases of the study (as described above) but the urinary phosphate increased when C_{PAH} was contemporaneously being determined, indicating suppression of TRP. Also, there were no other changes in PAH when TRP was observed, indicating that these levels of plasma phosphate augment C_{PAH}.

In conclusion, this study has shown that TRP does not become grossly abnormal until GFR is less than 30; TRP is depressed by the contemporaneous determination of C_{PAH}, and C_{PAH} is augmented by the contemporaneous determination of TRP by this technique.

51. VENTRICULAR FILLING PRESSURE IN ANEMIA

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This study was made to evaluate the importance of changes in ventricular filling pressure in the production of the increased cardiac output associated with anemia. Observations were made in 14 closed-chest dogs under morphine-chloralose anesthesia. Paired observations were made in each of three periods: (1) control; (2) oligemia produced by bleeding 20 ml. per kilogram; (3) oligemic anemia produced by further bleeding of 60 ml. per kilogram, with the blood removed replaced by an equal volume of isoneotic dextran.

Results.—Preliminary studies with 6 per cent dextran in 5 per cent dextrose showed the development of significant hyponatremia and hypocalcemia following dextran exchange, so that further studies were made with dextran in isotonic saline with added magnesium, calcium, and potassium. Mean blood volumes (Cr^{51}-tagged red cells) declined 15.5 per cent from control following bleeding and dextran exchange. Mean hematocrit ratio was 48.4, control; 48.9, oligemia; and 16.2 during oligemic anemia. During oligemia there was a consistent fall in right atrial transmural pressure, cardiac output, stroke volume, and right ventricular stroke work. During oligemic anemia, there was an increase of cardiac output, stroke volume, and right ventricular stroke work, but no change in right atrial transmural pressure as compared to the oligemic period.

Mean cardiac output was 1.935 L. per minute, control; 1.207 L. per minute during oligemia, $p < 0.001$; and 2.807 L. per minute during oligemic anemia. The mean cardiac output was significantly increased during oligemic anemia over control and oligemic periods ($p < 0.001$). The increase of cardiac output during oligemic anemia over control was due almost entirely to tachycardia, but the increase over oligemia was associated with increased right ventricular stroke volume and stroke work.

The results indicate that the cardiac output of dogs can be increased by acute anemia even though blood volume is significantly reduced. The increase of cardiac output, stroke volume, and right ventricular stroke work observed in these experiments was not associated with an increase of right atrial transmural pressure. Decrease in blood viscosity was not entirely responsible for the increased cardiac output during anemia. It is suggested that the increased cardiac output of anemia may be associated with increased cardiac contractility.

52. THE EFFECT OF N-(1-METHYL-2, 3, DI-P-CHLOROPHENYL-PROPYL)-MALEAMIC ACID (BENZMALECENE) ON SERUM LIPIDS AND LIPOPROTEINS

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Benzmalecene inhibits the incorporation of 1-C^{14} acetate and 2-C^{14} mevalonic acid into cholesterol by rat liver homogenates.

Benzmalecene was administered to two normocholesterolemic and one hypercholesterolemic subjects in doses of 1.0 to 1.5 Gm. per day for 7 to 12 days. Serum total and unesterified cholesterol, phospholipid, and triglyceride levels, and the cholesterol and phospholipid content of serum lipoprotein fractions isolated by differential ultracentrifugation were determined before, during, and after treatment. No changes were noted in serum transaminase (glutamic-oxaloacetic and glutamic-pyruvic) or van den Bergh levels. However, the compound produced nausea, which prompted one subject to refuse further medication after 12 days. No dietary restrictions were imposed and body weights remained unchanged.

Serum total cholesterol concentration in the hypercholesterolemic subject decreased 100 mg. per cent; in the normocholesterolemic subjects, 55 to 70 mg. per cent, due solely to reduction in esterified cholesterol. The cholesterol content of both the α lipoproteins (density $>$ 1.063 Gm. per milliliter) and the combined β and lower density lipoproteins (density $<$ 1.063 Gm. per milliliter) was reduced. However, after further fractionation of the lipoproteins of density $<$ 1.063 Gm. per milliliter in the hypercholesterolemic subject into components of densities $<$ 1.019 Gm. per milliliter and density $>$ 1.019 $<$ 1.063 Gm. per milliliter, it was noted that the cholesterol content of the lower density fraction ($<$ 1.019 Gm. per milliliter) had more than doubled.

Serum phospholipid and triglyceride levels increased in each subject. The increase in serum phospholipids was due to increase in the lipid phosphorus content of the lipoprotein fraction of density $<$ 1.019 Gm. per milliliter.

The effects of Benzmalecene administration on serum lipids and lipoproteins in human subjects may be summarized as follows: (1) decreased serum total and esterified cholesterol concentrations; (2) decreased cholesterol and phospholipid content of α (density $>$ 1.063 Gm. per milliliter) and β (density $>$ 1.019 $<$ 1.063 Gm. per milliliter) lipoproteins; (3) increased serum phospholipid and triglyceride concentrations; (4) absolutely and relatively increased cholesterol and phospholipid content of lipoproteins of density $<$ 1.019 Gm. per milliliter.

The compound possesses extraordinary lipid-altering properties which warrant further investigation. However, the increase in low density lipoprotein lipids may be an undesirable effect in view of the role such low density lipoproteins may play in atherogenesis.

53. MASSIVE, CONTINUED EXPOSURE TO BRUCELLA ABORTUS CAUSING THE BLOCKING PHENOMENON IN RABBITS

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The effects of hyperimmunizing rabbits with *Brucella abortus* antigen were studied from three aspects: the serum antibodies, the change in serum proteins, and the effect on a *Brucella melitensis* bacteremia. Hyperimmunity was pro-

duced by injecting intravenously 1.0 to 25.0 ml. of 1:5 diluted *Br. abortus* antigen 1 to 3 times a week for 10 to 23 weeks. Serial blood specimens were obtained by intracardiac puncture for the above studies.

Continued injection of large or small amounts of antigen resulted in a persistent elevation of the agglutinating antibody titer. Blocking antibodies, however, appeared only after weeks of massive antigen injections. Starch block fractionation of these sera revealed the blocking antibodies to appear early in the fast moving gamma globulin fractions. With repeated massive antigen injections the blocking activity progressively moved to reside after several weeks in the beta and alpha-2 globulin fractions. This same migratory phenomenon was observed in a man with acute brucellosis. Serial electrophoretic studies of the sera revealed no changes from that found in controls, unless repeated massive antigen injections were given. A marked rise in serum gamma globulins was then noted, which was not appreciably altered by specific antibody absorption. Hyperimmunized rabbits were found to handle a *Br. melitensis* bacteremia in the same manner as immunized animals; both groups of animals had drops in arterial brucellar counts to low plateau levels in one-half the time required by normals. This immune effect was specific. Brucellar immunized and hyperimmunized rabbits showed no alteration in the handling of *Escherichia coli* bacteremia from that found in normals.

These studies indicate a possible mode of genesis of the blocking phenomenon. Brucellar blocking antibodies are produced coincident with a marked hyperglobulinemia by repeated contact of the antibody-producing mechanism with large amounts of brucellar antigen. Prolonged antigenic stimulus, however, did not paralyze the production of agglutinating antibodies. No explanation for the change in electrophoretic mobility of the blocking antibody is possible at present.

54. METABOLIC EDEMA

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We have studied 15 patients with a clinical picture which we have called "metabolic edema." It occurs only in women and has not received attention proportionate to its very frequent occurrence. It has been referred to in the past as "cyclical edema" and "idiopathic edema of females." The present study was designed to investigate biochemical and psychological aspects of its pathogenesis.

Clinically, the cardinal manifestation is swelling, most often concentrated in the abdomen, arms, and legs, but sometimes apparent in the face and the rest of the body. Weight may increase by 2 to 8 kilograms within a day or two. Episodes last from a few days to a few weeks, and often terminate spontaneously by diuresis. They bear no recognizable relation to menstrual cycles. (Four of our patients were postmenopausal.) Routine clinical study reveals no evidence of the known cardiac, renal, hepatic, or nutritional causes of edema.

Eleven women were interviewed to obtain information concerning the relevance of life stresses and emotional disturbance to the development of the syndrome. In 8 cases the interviews were tape-recorded and later transcribed. All patients revealed serious life problems, resulting in emotional disturbance, present at the time of development of the edema. Nine of them unequivocally showed a psychological common denominator, different from that seen in other psychosomatic diseases. This can be epitomized in the attitude: "I am carrying too big a load; I wish somebody else would carry it." Eight patients emphasized care and responsibility for children in this "load."

Physiologically, it appears that the fluid retention is mediated in part through fluctuating but elevated levels of aldosterone released by the adrenal cortex. Measurement of 24-hour urinary output of this steroid showed values of 60 to 400 μg as compared to 3 to 10 μg per 24 hours for normal control subjects. Assays in patients during interedematous periods showed levels within the normal range. Biological assay of posterior pituitary antidiuretic hormone in 24-hour urine specimens on a few subjects revealed markedly elevated levels, so this hormone is probably also involved.

55. RENAL FUNCTION STUDIES IN PRIMARY ALDOSTERONISM WITH SUGGESTIVE EVIDENCE OF PHYSIOLOGIC REGULATION OF ALDOSTERONE SECRETION IN AN ADRENAL ADENOMA

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(INTRODUCED BY LESLIE ZIEVE, M.D.)

Studies were performed on a 59-year-old male with an aldosterone-secreting adenoma of the left adrenal cortex. GFR and ERPF were about half normal preoperatively. Bicarbonate reabsorption was elevated to approximately 3.0 mM per 100 ml. of glomerular filtrate initially. Following two weeks of Na restriction and K administration it had declined to about 2.7. Phosphorus clearance varied between 22 and 40 ml. per minute preoperatively. K excretion was consistently above 80 per cent of the filtered load. Three separate studies revealed excessive Na and Cl excretion. Water diuresis was produced by oral ingestion of 1,200 ml. of water followed by 200 ml. every 20 to 30 minutes. Following this procedure approximately 10 per cent of filtered Na and 15 per cent of filtered Cl were excreted in each clearance period at a time when the 24-hour Na excretion was about 100 mEq. Independent corroboration of this observation was found in the urine osmolality data. Specimens obtained approximately 1 hour after water loading revealed values of 83 and 97 mOsm. per kilogram of water. Osmolality of samples collected during clearance periods was 3 to 4 times as great. Reduction of GFR with hexamethonium resulted in complete reabsorption of Na and Cl. Postoperatively reabsorption of these ions was complete. However, interpretation of the data is complicated by the drastic reduction of GFR and ERPF.

Reduction of extracellular fluid volume is known to increase urinary aldosterone excretion. The converse situation has not been studied as well, but there is evidence that such procedures result in increased Na excretion. Na diuresis is known to occur as a result of simultaneous administration of vasopressin and water. ADH activity in our case is precluded by the low urine osmolalities before the clearance periods were started. The data are consistent with the hypothesis that saluresis may have resulted from a decrease in aldosterone secretion as a consequence of expansion of the extracellular space. This suggests that the adenoma was not physiologically autonomous but rather responded to a normal regulatory stimulus.

56. THE HEMODYNAMIC COMMON DENOMINATORS OF THE SYNDROME OF CHRONIC CONGESTIVE FAILURE

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In studies designed to quantitate circulatory dynamics in patients with varying circulatory loads, data have been obtained in our laboratory in 85 patients with hypo- and hyperthyroidism, systemic arteriovenous fistulas, anemia, beriberi, rheumatic heart disease, and myocarditis. Some of these pa-

tients had congestive failure clinically. Each group has been subdivided by the presence or absence of clinical failure and the hemodynamic data of all of those without failure compared with those of the subgroups with failure. The range of cardiac output observed at rest in the nonfailure category (1.88 to 7.38 L./min./M^2) did not differ from the failure category (1.95 to 8.93 L./min./M^2). During exercise, however, a significant increase in cardiac output and stroke volume occurred in each group in the nonfailure category in contrast to no change or a decrease observed in the failure category. The arteriovenous oxygen difference at rest ranged from 2.24 to 4.66 ml. per 100 ml. in the nonfailure and from 2.33 to 7.20 ml. per 100 ml. in the failure categories; a greater increase during exercise occurred in the latter category. The right atrial pressure was below 9 mm. Hg at rest and during exercise in the nonfailure groups, and above this level in the failure groups. No significant differences in mean brachial arterial pressures were observed. These data permit a description of the hemodynamic responses to chronic abnormalities of circulatory load and of the hemodynamic common denominators of the syndrome of clinical congestive failure which may result from a chronic excess of circulatory demand over cardiac output at varying levels of demand and of cardiac adequacy.

57. PHOSPHATE DISTRIBUTION AND P^{32} EXCHANGE IN THE ERYTHROCYTES OF INFANTS AND ADULTS

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The principal acid-soluble phosphates of the human erythrocyte are 2,3-diphosphoglycerate (2,3-DPG), adenosine triphosphate (ATP), adenosine diphosphate (ADP), and orthophosphate (P_i). These constituents were studied by the application of quantitative paper chromatography to the perchloric acid extracts of $NaH_2P^{32}O_4$-labeled erythrocytes of four newborn infants and four adults. The concentration and relative specific activity (R.S.A.) of each metabolite was determined serially during a 4-hour period of incubation at 37° C. The average total acid-soluble P of the fresh erythrocytes of the eight individuals was 1,450 μM per cent; 48.3 per cent was recovered as 2,3-DPG, 20.4 per cent as ATP, 5.4 per cent as ADP, and 5.4 per cent as P_i.

Comparison of the two groups showed the following: (1) Total acid-soluble P, 2,3-DPG, and ATP were lower, and P_i was higher in the fresh cells of the infants. (2) After incubation the relationships remained unchanged but the 2,3-DPG level decreased much more rapidly whereas the P_i rose much faster in the erythrocytes of the infants. (3) The relative rate at which R.S.A. developed in P_i and ATP was reversed in the infant and adult groups, the activity of P_i rising faster than that of the ATP in the adults. (4) Throughout the 4-hour incubation the R.S.A. of the intraerythrocyte components of the infants was significantly lower than that of the adults.

58. CLINICAL APPLICATION OF THE RADIOACTIVE TRIOLEIN TEST IN DIFFERENTIAL DIAGNOSIS OF PANCREATIC LESIONS

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Since its inception in 1955, the I^{131}-labeled triolein test has seen success as an experimental method of determining the relative amounts of pancreatic lipase excreted by the normal and diseased organ. We have used the triolein

test and I^{131}-labeled oleic acid test in the study of normal patients, carcinoma of the head of the pancreas, chronic pancreatitis, and in jaundiced patients who did not have related pancreatic problems. The tests were performed on 151 patients.

Volunteer patients who had no gastrointestinal disorders were tested and this established our criteria for normal uptake curves. Fifty-one normal patients were tested, 22 carcinoma of the head of pancreas, 26 chronic pancreatitis, 14 medical jaundice, 12 other surgical jaundice patients, and 14 miscellaneous cases. The diagnoses in the pathologic conditions were established either at autopsy, at surgery, or on the basis of clinical follow-up and recovery. We found that patients suffering from pancreatic disorders showed markedly different uptake curves, as well as the actual percentage of uptake, than the normal or jaundiced patients. The triolein uptake peak as well as the type of curve of chronic pancreatitis was shown to be different from those of patients suffering with carcinoma.

With aliquots of whole blood samples taken at 2, 4, 5, and 6 hours after the test meal was given, it could be shown that normal patients had a mean uptake of 4.1 per cent with mean 5-hour peaks. Carcinoma patients had absolutely flat uptake curves with the 5-hour mean at 0.96 per cent. In contrast to this, chronic pancreatitis showed a low peaked curve with 1.72 per cent mean uptake at 5 hours. In the study of patients with medical and surgical jaundice it was shown that they had "normal" curves with 5-hour peaks at 3.02 and 3.66 per cent, respectively. These and the other figures in the study were proved statistically significant.

In conclusion, we feel that we have been able to make the triolein test a simple yet accurate one for the differential diagnosis of pancreatic disease and, especially, carcinoma of the head of the pancreas.

59. STUDIES ON THE MECHANISM OF EDEMA PRODUCED BY PROLONGED INFUSION OF NOREPINEPHRINE

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Prolonged infusion of epinephrine or norepinephrine is known to reduce plasma volume, increase hematocrit, and sometimes produce edema and shock. The mechanism of these changes is unknown. Previous studies have shown that norepinephrine may produce a per cent increase of venous resistance which is greater than the per cent increase of artery resistance (Circ. Res. 5 : 247, 1957). This suggested the following mechanism: venous constriction → rise of capillary hydrostatic pressure → increased in rate of outward filtration → reduced plasma volume and edema. In order to test this hypothesis, norepinephrine was infused into the brachial or mesenteric arteries of the dog for periods up to 60 minutes while measuring systemic arterial pressure, paw or mesenteric small venous pressure, cephalic or portal venous pressure, and weight change of limb or gut loop.

Infusion into the brachial artery at rates between 0.4 and 30.4 μg per minute immediately decreased small venous pressure and limb weight. In 8 of 19 experiments, this was shortly followed by rise of small venous pressure to levels as high as 44 mm. Hg. This was associated with a gradual and steady gain in limb weight to levels also above the control value. In the remaining instances, weight did not increase but neither was there a sustained change of small venous pressure. Upon stopping the infusion, small venous pressure rose as high as 64 mm. Hg. In 3 of 8 gut experiments, infusion rates from 1 to 27 μg per minute

elevated small venous pressure and gut weight. In the remaining experiments, weight did not increase but neither was there a sustained rise of small venous pressure.

These studies show a positive correlation between elevations of small venous pressure and weight. Since capillary pressure in open beds cannot be lower than small venous pressure, the larger increases of small venous pressure were almost certainly associated with rise of capillary pressure. The steadiness of the weight gain indicates that the gain represented increase of interstitial fluid rather than vascular volume. Therefore, it seems probable that in some instances norepinephrine constricts veins proportionately more than arteries resulting in rise of capillary hydrostatic pressure and hence outward filtration of fluid.

60. THE DEFENSE OF TWO MECHANISMS FOR THE PRODUCTION OF ADRENAL HYPERPLASIA RESULTING IN HYPERADRENOCORTICISM (CUSHING'S SYNDROME)

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Evidence favoring primary hypothalamic or pituitary etiology of hyperadrenocorticism includes: (1) basophilic pituitary adenoma present in 41 per cent of autopsied cases; (2) remission with pituitary irradiation (25 to 50 per cent); (3) remission after hypophysectomy (3 cases); (4) suppression of corticosteroid excretion with fludrocortisone (50 to 100 per cent); (5) development of pituitary adenoma after subtotal adrenalectomy; (6) production of anatomic and microchemical adrenal changes similar to adrenal hyperplasia in normal patients given ACTH.

The concept that the primary defect is adrenal is favored by: (1) failure to demonstrate elevation of ACTH in plasma; (2) marked hyperresponsiveness of plasma and urinary 17 OHCS to ACTH; (3) associated hyperaldosteronism; (4) frequency of irradiation failures. The clinical and laboratory findings and treatment response in two patients suggest two mechanisms as follows.

CASE 1 (pituitary).—(1) Hypercorticism, male, 6; (2) no evidence pituitary or adrenal tumor; (3) urinary 17 OHCS rose (19-24 to 40-55 mg. per day) after ACTH; (4) 17 OHCS suppressed by fludrocortisone, 4 mg. per day; (5) resection left (96 Gm.) and 2/3 right adrenal (4 Gm.) produced chemical and clinical remission, maintenance corticoid not required; (6) progressive dermal pigmentation started one month after surgery, was uninfluenced by oral corticoid trial; (7) urinary and plasma 17 OHCS normal and not now responsive to ACTH; (8) basophilic adenoma developed, enlarged sella, caused bitemporal hemianopsia, and required resection; (9) preoperative plasma ACTH definitely elevated (courtesy Dr. Don Nelson).

CASE 2 (adrenal).—(1) Hypercorticism, male, 23; (2) no evidence of tumor; (3) plasma 17 OHCS rose (16 to 146 meg. per cent) with ACTH, not suppressed with fludrocortisone 6 mg. per day; (4) resection, all of left (5.4 Gm.), 60 per cent right adrenal (5.4 Gm.) failed, clinical symptoms and hyperresponsiveness to ACTH persisted; (5) 5,000 r delivered to pituitary failed to induce remission in 5 months; (6) reoperation demonstrated 2 Gm. remnant had increased to 20 Gm.; (7) removal resulted in cessation of steroid secretion.

Recent medical fashions would tend to favor a primary hypothalamic or pituitary etiology and exclude an intrinsic adrenal abnormality. It is concluded that both mechanisms are possible.

61. ACID-BASE ABNORMALITIES ENCOUNTERED WITH THE GIBBON-MAYO PUMP USING BLOOD FLOW RATES OF 2-2.4 LITERS PER SQUARE METER OF BODY SURFACE PER MINUTE

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(INTRODUCED BY JOHN R. SMITH, M.D.)

It was noted that the venous blood of postoperative patients who had undergone repair of congenital cardiac malformation using the Gibbon-Mayo pump showed profound acidemia while the arterial oxygen saturation (earpiece oximeter) was well above 90 per cent. Their clinical appearance seemed much worse than the arterial oxygen saturation would indicate.

It was because of these discrepancies that the following studies were undertaken: (1) Thirty patients who had surgical repair of cardiac defects using extracorporeal support were studied. The following blood constituents were determined: pH, CO_2, lactic acid, sodium, potassium, calcium, phosphorus, chloride, oxygen saturation and total and fractional proteins on the (a) donor blood, (b) blood in the pump prior to bypass, (c) arterial and venous blood of the pump at the end of bypass, (d) femoral artery and vein blood of the patient 4 hours postoperatively. (2) The postoperative A-V difference studies were also carried out on 10 hypothermia patients and upon a similar number of patients who had an unilateral thoracotomy for the repair of a patent ductus arteriosus or coaretation of the aorta.

Conclusions.—(1) The acidemia of the pump blood before and during bypass was mild and was due to the method of collection and processing of the donor blood. This was primarily due to stasis, glycolysis, and dilution with saline. (2) The A-V difference of the pump blood at the end of bypass was in the normal range, indicating good circulation during bypass. (3) The A-V difference of the patient's blood 4 hours after bypass was marked, as is shown by findings in a typical case.

	Arterial Blood		*Venous Blood*	
pH	7.34		7.22	
CO_2	15.9	(whole blood) mEq./L.	20.2	(whole blood) mEq./L.
pCO_2	33	mm. Hg	54	mm. Hg
Calculated acid excess	4.0	mEq./L.	4.0	mEq./L.
Lactic acid	5.4	mEq./L.	5.8	mEq./L.
Oxygen saturation	91.9	%	28.0	%

Similar studies in the posthypothermia and unilateral thoracotomy patients revealed only moderate changes. (4) These A-V differences indicate poor circulation in the extremity from which these samples were drawn. This could be accounted for by (1) low cardiac output, (2) local vasospasm, or a combination of both.

Studies are presently in progress to resolve this problem.

62. HEMODYNAMIC RESPONSE TO EXERCISE IN DEXTRAN-INDUCED HYPERVOLEMIA AND IN ACUTE GLOMERULONEPHRITIS

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High cardiac outputs and elevated filling pressures have been reported from this laboratory in a group of patients with acute glomerulonephritis, some of whom had evidence of circulatory congestion. It was difficult to decide whether these hemodynamic changes were only due to hypervolemia, or whether heart failure was also present.

To investigate this problem, the hemodynamic response to exercise was studied by heart catheterization in the following subjects: (a) 6 normal controls; (b) the same 6 normals at the end of an intravenous infusion of 1,200 ml. of 6 per cent dextran in water (30 ml. per minute); (c) 4 patients with acute glomerulonephritis. Dextran infusion in resting normals produced the following mean increments: cardiac index (CI) +1.18 ± 0.38 L./min./M² BSA ($p < 0.05$); pulmonary "capillary" mean pressure (PC) +8.7 ± 2.1 mm. Hg ($p < 0.01$); brachial artery mean pressure (BA) +5.0 ± 8.1 mm. Hg ($0.6 > p > 0.5$).

On exercise the following mean changes were observed in the control group: O_2 consumption +384 ± 22 ml./min./M²; CO +741 ± 76 ml./100 ml. increase in O_2 consumption/min.; PC +3.2 ± 0.32 mm. Hg ($p < 0.001$); left ventricular work index (LVWI) +4.20 ± 0.66 Kg. M/sec./M² ($p < 0.01$); left ventricular stroke work index (LVSWI) +11.0 ± 6.7 Gm. M/beat/M² ($0.1 > p < 0.2$). The induction of hypervolemia with dextran in these normals was associated with an exercise response in every manner similar to that with normovolemia.

The glomerulonephritis patients at rest had elevated mean CI (4.61 ± 0.21 L./min./M²), mean PC (13.5 ± 1.8 mm. Hg), and mean BA (132.7 ± 11.1 mm. Hg). On exercise, the O_2 consumption was increased +401 ± 72, CO +648 ± 62, PC +8.5 ± 2.7, LVWI +4.2 ± 0.51. These data do not differ significantly from the exercise data in the dextran-infused normal.

In all groups the increased CO was produced almost exclusively by cardiac acceleration and there was no correlation between filling pressure and stroke work. As judged by the cardiac output exercise response per 100 ml. oxygen consumption increment and the left ventricular work per minute exercise response, the status of the heart in acute glomerulonephritis does not differ significantly from the normal with acutely induced hypervolemia.

63. ALDOLASE, ISOCITRIC DEHYDROGENASE AND MALIC DEHYDROGENASE IN GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENT ERYTHROCYTES

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The genetic defect of glucose-6-phosphate dehydrogenase (G-6-PD) in the erythrocytes of approximately 10 per cent of American Negroes is not associated with any impairment of the viability of these cells unless they are exposed to primaquine and other agents. The apparent reason for this absence of a detrimental effect is the fact that utilization of glucose by the red cell takes place mainly (80 to 90 per cent) via the Embden-Meyerhof cycle. Nevertheless, it

has been suggested that as a compensatory mechanism, enzymes of the Embden-Meyerhof cycle may have increased concentration in these erythrocytes. It is also possible that the tricarboxylic acid cycle, usually not operating in mature erythrocytes, may function in the G-6-PD deficient cells and thus contribute to the regeneration of TPNH. As part of a study of this problem the enzymes aldolase (Ald.), isocitric dehydrogenase (ICD), and malic dehydrogenase (MD) were measured in the erythrocytes of 17 Negroes who were detected to have G-6-PD deficient red cells during the screening of the hospital population for carriers of this trait. The mean values (± S.D.) of the enzymes in hemolysates from these subjects were as follows (calculated per gram of hemoglobin): G-6-PD 0.24 (± 0.29) μM of TPNH (method of Horecker and Kornberg), Ald. 0.94 (± 0.22) μM of fructose diphosphate (modified method of Lowry), ICD 1.1 (± 0.42) μM of TPN (method of Wolfson and Williams-Ashman), MD 190 (± 45) μM of DPN (method of Ochoa). The respective values for normal red cells from 9 Negroes were 5.1 (± 2.5), 1.1 (± 0.2), 1.7 (± 0.48), and 201 (± 61). Except for the G-6-PD values ($p < 0.001$), no statistically significant difference was detected in the determined enzyme values between the carriers of this abnormality and normals. These results for the aldolase are at variance with the results reported by Schrier and associates, who used a different method and found that the aldolase activity was consistently increased by approximately 30 per cent in all subjects tested. Possible causes for this divergence will be discussed.

It appears that the maintenance of normal metabolic functions of the G-6-PD deficient red cells under stress-free circumstances can be adequately secured without a compensatory increase in glycolytic enzymes.

64. A COMMUNITY AND FAMILY STUDY OF ASIAN INFLUENZA

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(Introduced by F. M. Davenport, M.D.)

During the epidemic of Asian influenza in 1957 a unique opportunity was presented to ascertain the influence of density of exposure and of relative immunity upon the incidence of clinical and subclinical infection in a well-defined population resident in Tecumseh, Michigan. This opportunity occurred because there was ample time to formulate plans before the epidemic struck, since incidence was high and noninfluenza respiratory disease could be readily distinguished, and because the prevailing virus was antigenically so distinctive that a presumptive diagnosis of recent infection could be made using a single serum sample. To measure clinical infection, the attack rate calculated from reports of daily illness in 1,495 households, consisting of 5,233 individuals, was used. Subclinical incidence was estimated by subtracting that rate from a prototype serologic attack rate obtained by calculating the difference in the frequency of Asian antibody in pre- and postepidemic collections of sera drawn from non-respiratory patients at the University of Michigan Hospital. The influence of density of exposure upon illness was studied by comparing age- and sex-specific attack rates in households of different size and of different composition by age, sex, and number. Age-specific clinical and subclinical attack rates in all families were highest in childhood and declined progressively thereafter. A higher proportion of the infections of the older age group was subclinical. The clinical attack rate at all ages, including adults, was higher in households containing children and in households of the larger sizes. In families with children, adult females became ill more frequently than adult males. In families without children, the attack rate tended to be higher in males. The relative immunity of the older segments of the population, conferred by prior experience with

multiple antigenic variants of influenza A virus, was not abolished, even though their sera, in general, did not contain detectable Asian antibody. The results of this study illustrate the many factors that influence the attack rate of influenza.

65. THE MECHANISM OF HEMOLYSIS IN PAROXYSMAL COLD HEMOGLOBINURIA AS A MODEL SYSTEM FOR STUDYING THE MECHANISM OF ACTION OF HUMAN COMPLEMENT

CARL F. HINZ, JR., M.D., MARY E. PICKEN, B.S. (BY INVITATION), AND IRWIN H. LEPOW, PH.D., M.D. (BY INVITATION), CLEVELAND, OHIO

Hemolysis of erythrocytes by the Donath-Landsteiner (D-L) hemolytic antibody, causing paroxysmal cold hemoglobinuria, requires complement in both the initial cold and the subsequent warm phase of hemolysis. The role of complement in immune hemolytic reactions has been recognized since the work of Ehrlich before 1900, but the mechanism by which complement causes hemolysis remains unknown. Recently it has been proposed that complement action is initiated by the first component of complement (C′1), which exists in serum as a proenzyme, and is activated by antigen-antibody complexes to have esterase properties which hydrolyze certain synthetic amino acid esters.

The present studies, utilizing human serum complement and erythrocytes and partially purified D-L hemolysin, permit a separation of the initial and final phases of complement action because of the cold-warm nature of the D-L reaction. They indicate that immune hemolysis is initiated by C′1, for C′1 is the only component of complement required in the cold phase, whereas the other three components participate in the warm phase; and they suggest that the effect of C′1 may be due to its enzymatic properties. The results show that (1) the cold phase is completed successfully if fresh serum is present to supply complement; (2) the cold phase is not completed if C′1 is inactivated in serum by heating or is removed from serum by precipitation during dialysis at low ionic strength; inactivation of other components of complement does not interfere with the cold phase; (3) partially purified C′1 either as proenzyme or activated enzyme (C′1-esterase) effectively replaces fresh serum in the cold phase; (4) the effect of C′1-esterase is blocked completely by an inhibitor isolated from serum, which acts against the esterase properties; (5) C′1 is not required in the warm phase but the other three components of complement are.

These observations are consistent with the hypothesis that in the presence of the erythrocyte-antibody complex, C′1 is activated from the naturally occurring proenzyme to C′1-esterase which initiates the hemolytic effect of complement. This system affords a model for studying the mechanism of action of human complement on human erythrocyte-human antibody complexes.

66. EFFECTS OF A PLANT ALKALOID, VINCALEUKOBLASTINE, IN HUMAN BEINGS

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Extracts of *Vinca rosea* produce bone marrow depression in animals. Several alkaloids have been isolated from the plant. One, vincaleukoblastine (Eli Lilly and Company, Compound 29060-LE), not only causes marrow depression but has produced 50-150 per cent prolongation of life in DBA/2 mice grafted with leukemia P-1534 (Johnson, Wright and Svoboda). This study

concerns the effect of this agent in human beings suffering from a number of advanced neoplastic disorders. Single and repeat courses of the alkaloid have been administered once a day (0.1 to 1.5 mg. per kilogram) for periods ranging from 1 to 11 days.

Twenty patients had received the drug when the abstract was submitted. All cases which can be evaluated have experienced a drop in peripheral total leukocyte count, with the granulocytes affected most. Hemoglobin, erythrocytes, and platelets are generally affected slightly or not at all. Some patients have exhibited a rise in reticulocyte and platelet counts concomitant with a decrease in abnormal white blood cells. Response has followed a total dose of 0.45 to 2.25 mg. per kilogram.

Toxic effects include anorexia, nausea, stomatitis, mental depression, weight loss, local phlebitis, epilation, and marrow depression.

Complete hematologic remission has been achieved in acute lymphocytic and monocytic leukemia. In those situations where hematologic remission was not achieved, tumor cell infiltrates have decreased in size.

The mode of action of the drug is unknown. Tissue culture studies indicate that the alkaloid produces metaphase arrest (Palmer, Johnson, Warren, Simpson, and Livengood). Some patients had previously been treated with other agents, and cross-tolerance has not been seen.

The effect of vincaleukoblastine on a broad spectrum of human tumors is under investigation. Dose-response relationships and clinical data will be presented.

67. THE EFFECT OF ACETYL STROPHANTHIDIN ON PERIPHERAL VENOUS TONE IN MAN

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Some investigators believe that digitalis causes peripheral venous constriction. Others suggest that the drug induces venous dilatation. Neither hypothesis is supported by measurements of venous tone in normal human subjects. The experiments were done on 5 normal men. Venous pressure-volume curves were obtained from the left forearm by a plethysmographic method (J. Clin. Invest. 37: 956, 1958). Pressure was measured in the antecubital vein of the dependent right arm. Arterial pressure was measured with a cuff on the arm proximal to the venous needle. The electrocardiogram was monitored continuously with an oscilloscope and recorded intermittently. After control values were obtained, 0.75 mg. of acetyl strophanthidin diluted in saline was injected intravenously. An additional 0.375 mg. was given at 5 minutes and again at 10 minutes after the initial injection. Observations were continued intermittently for 45 minutes after the final dose.

Heart rate averaged 69 beats per minute during control periods and 58 after the drug. Mean arterial pressure increased 9 mm. Hg. Venous pressure did not change appreciably. No major alterations occurred in the electrocardiogram. Venous tone increased in 4 experiments and remained unchanged in 1; the forearm venous blood volume at a transmural venous pressure of 30 mm. Hg averaged 4.1 ml. per 100 ml. of tissue during control periods and fell to 3.7 ml. after drug administration. The change averaged 0.4 ml. ($p < 0.02$).

Preliminary experiments were done on 4 subjects. In these the drug was given in multiple small doses over periods of 15 to 20 minutes. In 1 the total dose was only 1.2 mg. Venous tone increased in 1 and remained essentially un-

changed in the other 3 tests. When all 9 experiments were grouped together, the average change in venous volume at a transmural pressure of 30 mm. Hg was a decrease of 0.3 ml. per 100 ml. ($p < 0.02$).

In these experiments intravenous administration of acetyl strophanthidin induced mild venous constriction. Venous dilatation was not observed.

68. STUDIES ON THE HETEROZYGOUS CARRIER IN GALACTOSEMIA

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(Introduced by Walter A. Rambach, M.D.)

Galactosemia is a hereditary disorder of carbohydrate metabolism characterized by vomiting, diarrhea, jaundice, poor weight gain, and malnutrition during early infancy. The condition is transmitted by an autosomal recessive gene and results from a deficiency of the enzyme galactose-1-phosphate uridyl transferase.

The purpose of the present communication is to report on the measurement of galactose-1-phosphate uridyl transferase activity among members of 12 galactosemic families. In each instance, the enzymatic content of erythrocytes was assayed by four different approaches: (1) the uridine diphosphoglucose consumption test as described by Anderson and associates (J. Lab. & Clin. Med. 50: 469, 1957); (2) the same test using shorter incubation time and higher substrate concentrations as described by Bretthauer and associates (Proc. Nat. Acad. Sc. 45: 328, 1959); (3) a Warburg measurement of oxygen consumption using supplementary phosphoglucomutase and glucose-1,6-diphosphate as described by Kirkman and Bynum (Ann. Human Genetics 23: 117, 1959); and (4) the same oxygen consumption test without the supplementary compounds. A decrease of galactose-1-phosphate uridyl transferase activity was noted among the heterozygotes, by use of all four of these methods. The relative sensitivity and accuracy of each method will be discussed. We found that a small, but significant, amount of enzyme activity was detectable among many of the individuals classified as homozygotes.

In the course of these studies, several observations were made regarding the maintenance of zero order kinetics in the galactose-1-phosphate uridyl transferase system. (1) In our systems, there appears to be a 10 to 15 minute lag before the initiation of observable oxygen uptake and this has to be taken into consideration when calculating enzyme activity. (2) In the course of measuring maximal observable activity, it was found that by increasing the concentration of UDPG tenfold, only a 50 per cent increase of enzyme activity was noted. (3) Finally, when 5 μM of Gal-1-P and 3 μM of UDPG were used in the standard test, the maintenance of zero order kinetics was not dependent upon the use of supplementary phosphoglucomutase and glucose-1,6-diphosphate as previously described.

69. A CASE OF DIFFUSE DESMOPLASTIC ENDO- AND PERIPHLEBITIS WITH UNILATERAL PULMONARY ARTERIOSCLEROSIS

Scott R. Inkley, M.D., and George Richard Abbott, M.D., Cleveland, Ohio
(Introduced by Dieter Koch-Weser, M.D.)

A disease process which not only has not been previously reported in the literature but which produced very unusual changes in the pulmonary circulation is described. The lesion was characterized by a dense diffuse fibrous con-

nective tissue growth following the great veins of the thorax and abdomen. This same desmoplastic process was also found in the posterior mediastinum, parietal pleura, retroperitoneal tissue, pericardium, and mesentery.

A very interesting autopsy finding was marked pulmonary arteriosclerosis limited to the lung whose venous drainage had gradually been obstructed by the connective tissue growth. This case affords a most unusual chance to speculate on the etiology of pulmonary arteriosclerosis in the light of the many studies that have been carried out in laboratory animals in an effort to produce pulmonary arteriosclerosis.

70. PULMONARY VASCULAR CHANGES IN RABBITS PRODUCED BY INTRAVENOUS THROMBOPLASTIN

Scott R. Inkley, M.D., Simon Koletsky, M.D., and Louis Gillespie, M.D.,
Cleveland, Ohio
(Introduced by Robert H. Ebert, M.D.)

This study was designed to determine the pulmonary vascular changes of rabbits following intravascular clotting in the peripheral venous circulation. Two series of 20 rabbits each were given intravenous injections of equal amounts of homologous thromboplastin at regular intervals for a 6-month period. One series received thromboplastin which had a high clotting activity and the other series of rabbits was given material which had been deactivated by the method of Quick. Some animals died shortly after injection of the material following an anaphylactic type of reaction, the remainder were sacrificed after 27 injections.

Changes in the pulmonary vascular bed were then evaluated by the double blind technique and graded by the pathologist before the key was broken. Rabbits receiving active material showed significant intraluminal changes consisting of thrombus formation, re-endothelization of the thrombus, and proliferative intimal fibroplastic reaction which is later seen as plaques of intimal fibrosis. The findings of this study confirm the reports of others using similar techniques and reaffirm the possibility that intravascular clotting may be one step in the development of certain types of arteriosclerosis.

71. THE EFFECT OF SMALL DOSES OF CORTISONE UPON URINARY 17-KETOSTEROID FRACTIONS IN WOMEN WITH HIRSUTISM AND OVARIAN DYSFUNCTION

William McK. Jefferies, M.D., and Richard P. Levy, M.D. (By Invitation),
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We have reported previously that women with hirsutism, often of mild degree, and ovarian dysfunction, manifested by irregular menses or amenorrhea, frequently respond favorably to the continual oral administration of small doses of cortisone or hydrocortisone. Abnormal patterns of urinary 17-ketosteroid (17-KS) fractions have been reported in such patients by several laboratories. Utilizing a technique for fractionating urinary 17-KS consisting of extraction of the conjugates, gentle hydrolysis of the glucuronidates with β-glucuronidase and of the sulfates with ethyl acetate, and gradient elution from an alumina column, four fractions with mobilities corresponding to dehydroepiandrosterone (DHA), androsterone (A), etiocholanolone (E), and 11-oxygenated-17-ketosteroids (11-oxy-17-KS) were separated in a reproducible fashion. The fractions of 3 normal, nonhirsute, regularly ovulating women with proved fertility showed the following ranges of values per 24 hours: DHA, 0.6 to 1.5 mg.; A, 0.5 to 1.8 mg.; E, 2.5 to 4.6 mg.; and 11-oxy-17-KS, 0.6 to 1.1 mg.

17-KS as determined by a standard method employing hydrolysis with hot acid (a-KS) were between 9.1 and 10.6 mg. per 24 hours. Of 4 patients with hirsutism and ovarian dysfunction, whose a-KS were between 13.8 and 21.6 mg., all had a higher A fraction (range 2.5 to 6.1 mg.), 2 had higher DHA values (range 0.3 to 4.5 mg.), and one had a higher E excretion (range 4.2 to 6.2 mg.). In all 4 patients, the administration of cortisone or hydrocortisone in doses of 5 mg. every 8 hours resulted within 2 months in a decrease in excretion of these fractions to levels exhibited by the normal subjects. In 3 of the 4 cases the steroid was administered in double-blind placebo studies. In no instance was administration of the placebo accompanied by reduction to the levels excreted by the normal subjects.

The results indicate that even small doses of cortisone or hydrocortisone can restore an abnormal 17-ketosteroid excretion pattern to normal. The amounts required are less than those believed to be secreted by normal subjects.

72. EXPERIMENTAL BASIS FOR CLINICAL EVALUATION OF ANTITUMOR PRINCIPLES DERIVED FROM VINCA ROSEA LINN

I. S. Johnson, Ph.D., H. F. Wright, M.A., and G. H. Svoboda, Ph.D., Indianapolis, Ind.

(Introduced by K. G. Kohlstaedt, M.D.)

The plant, *Vinca rosea* Linn, has enjoyed a popular reputation in indigenous medicine in various parts of the world. As early as 1910 it was described as having wound healing properties; in Europe related species have been used for the proprietary suppression of the flow of milk; in the British West Indies it has been used to treat diabetic ulcers; and in the Philippines it has been reported as being an effective oral hypoglycemic agent. This latter reported use prompted a phytochemical investigation of this drug in our laboratories. While the sample at hand gave little activity in this area, certain extracts submitted for routine cancer screening in December, 1957, were discovered to give interesting and in some cases profound antileukemic activity against the P-1534 lymphocytic leukemia in DBA/2 mice. Since other clinically useful antitumor agents have been developed in these laboratories (Johnson and Wright) using activity against this tumor as a guide, an extensive investigation was undertaken to determine the active ingredient(s).

Early in the course of this investigation it became evident that the major antileukemic activities were restricted to various alkaloidal entities and were primarily located among the weak bases. During the course of this work another group (Noble, Beer, and Cutts) reported the procurement of an alkaloid from *Vinca rosea* which they named vincaleucoblastine and which was characterized biologically by causing a profound depression of normal rat bone marrow. Subsequently, marked inhibition of the P-1534 leukemia was demonstrated with this compound in both Noble's and our laboratories. The results of studies with various extracts and alkaloids (including vincaleucoblastine) in a series of experimental neoplasms and tissue culture systems will be presented. This work has provided a basis for the clinical evaluation of a new class of antitumor compounds (Hodes, Rohn, and Bond).

73. SELECTIVE CINECARDIOANGIOGRAPHY IN THE EVALUATION OF MITRAL AND AORTIC VALVULAR DISEASE

WALTER E. JUDSON, M.D., AND EUGENE KLATTE, M.D. (BY INVITATION), INDIANAPOLIS, IND.

Differentiation and quantitation of predominant stenosis or regurgitation in mitral and aortic valvular disease by hemodynamic alterations and indicator dilution curves have not been invariably successful. Selective cinecardioangiography appeared to be a technique which would provide visual evidence of the dynamic function and competency of the atrioventricular and aortic valves. A No. 8 Lehman catheter with an inner wire stylus and flexible spring extending approximately 3 cm. beyond its tip was introduced into the femoral artery by percutaneous needle puncture and guided fluoroscopically into the aortic valvular area, where by careful manipulation it was usually possible to enter the left ventricle. Selective injections of contrast media were made in the left ventricle and ascending thoracic aorta with the course of the radiopaque material recorded by high-speed motion picture photography. After injection into the left ventricle, mitral regurgitation was demonstrated by the reflux of streaming contrast media through the incompetent mitral valve into the left atrium. Similarly visual proof of incompetency of the aortic valve was confirmed by regurgitation of the radiopaque media into the left ventricle following its rapid injection into the ascending aorta immediately distal to the aortic valve. The reflux of the radiopaque material through normal competent mitral and aortic valves or dominant stenosis was not observed.

The application of this technique in conjunction with catheterization of the right and left sides of the heart has proved especially valuable in the further differentiation of predominant mitral and aortic stenosis from incompetence, in the demonstration of gross mitral regurgitation in the absence of a loud systolic murmur, and in the separation of "re-stenosis" from progressive mitral incompetence following commissurotomy.

74. AN ALTERATION OF INTERMEDIARY CELLULAR METABOLISM RELATED TO PYRUVATE AND ORGANIC PHOSPHATE IN THE EXPERIMENTAL NEPHROTIC SYNDROME

ELIZABETH KAISER, PH.D. (BY INVITATION), MARCO PICCARDO, M.D. (BY INVITATION), AND JACK METCOFF, M.S., M.D., CHICAGO, ILL.

Except for gross changes in protein and lipid metabolism, specific alterations of intermediary metabolism have not been described in the nephrotic syndrome induced in rats by injection of 6-dimethylaminopurine riboside, an aminonucleoside structurally related to adenosine. The experimental disease closely simulates the clinical, pathological, and biochemical features of the syndrome observed in human beings. Previous studies on weanling nephrotic rats indicated excessive intracellular H_2O and Na, but reduced cellular nitrogen and K, and suggested an unusual aberration in K metabolism. Since cellular concentrations of K and Na may influence the dephosphorylation of phosphoenolpyruvate to pyruvate with its associated energy yield (as ATP) and subsequent pathways of pyruvate utilization (via alanine and amino acid synthesis or acetyl Co A and lipid synthesis), systematic exploration of cellular electrolytes and organic intermediates relating to pyruvate have been initiated.

The present study compares the pyruvate uptakes of isolated diaphragms, the organic and apparent inorganic phosphate contents of muscle, and the concentrations of free amino acids in kidney and liver homogenates of 8 weanling,

male Rockland Farm strain nephrotic rats with those of pair-fed controls. The data reveal a statistically significant increase of in vitro pyruvate uptake by the isolated diaphragms (7.1 versus 5.3 μM per minute per gram noncollagenous nitrogen); but a marked reduction in muscle organic phosphate (6.5 versus 15.5 mM per 100 Gm. dry fat-free solids) kidney alanine (162 versus 539 μM per 100 Gm. DFFS) and liver threonine contents (175 versus 570 μM per 100 Gm. DFFS) of the nephrotic rats versus their pair-fed controls.

The data indicate a defect of intermediary metabolism involving cellular phosphate and pyruvate transformations in the experimental (aminonucleoside) nephrotic syndrome of rats.

75. PHYSIOLOGIC DIFFERENCES BETWEEN STEROID AND BACTERIAL PYROGENS

ATTALLAH KAPPAS, M.D., PAUL GLICKMAN, M.D. (BY INVITATION), ROBERT H. PALMER, M.D. (BY INVITATION), AND BELA RATKOVITS (BY INVITATION), CHICAGO, ILL.

A number of steroid metabolites of the pregnane (5β-H) type, derived from endogenous adrenocortical and gonadal hormones, have been shown to consistently provoke fever following their intramuscular and intravenous administration to man. The biologic properties of these thermogenic compounds have been examined and significant differences have been noted between their actions and those of bacterial pyrogens. These differences include the following: Steroid pyrogens fail to provoke fever in a number of laboratory animals, including primates, indicating a high degree of species specificity of their thermogenic activity. There is a consistent lag period of several hours between the administration of steroid pyrogens and the initiation of fever in man, and this lag period is observed following the intravenous as well as the intramuscular administration of these agents. Rapid intravenous injection of steroid pyrogens does not provoke fever while prolonged infusion of these compounds is thermogenic. These steroids produce leukocytosis without initial leukopenia in man. Repeated administration of these steroids does not result in the development of tolerance to their fever-producing activity. The release of a secondary pyrogen into the blood stream in man, comparable to the endogenous thermogenic substance appearing in the blood stream of animals following bacterial pyrogen administration, cannot be demonstrated in plasma withdrawn during the development and peak of steroid pyrogen fever.

The fever produced by the administration of bacterial pyrogens to animals has served as the experimental model for elucidating the mechanism of fever in man. The present studies with steroid pyrogens in human beings indicate significant differences between the biologic action of these substances and bacterial pyrogens, and these physiologic differences may imply different mechanisms of fever production. The endogenous origin of these pyrogenic steroids, their apparent species specificity, their availability as pure compounds through chemical synthesis, and the physiologic properties noted above afford unique advantages for their use in studying the pathogenesis of fever directly in man.

76. PLASMA CLEARANCE OF INDUCED HYPERLIPEMIA: THE EFFECT OF REPEATED DAILY INTRAVENOUS ADMINSTRATION OF A FAT EMULSION

FRED KERN, JR., M.D., HARBANS SINGH, M.D., AND GILBERT S. MCDONOUGH, M.D., DENVER, COLO.

(INTRODUCED BY GORDON MEIKLEJOHN, M.D.)

Many of the factors which determine how much fat can be safely administered are poorly understood. The rate at which fat leaves the plasma after intravenous injection may be an indication of the ability of the organism to metabolize and/or store fat. The slowing of clearance rate as fat is repeatedly administered would suggest that the limit of tolerance is being reached. Accordingly a study was undertaken to observe the effect of daily administration of a nontoxic fat emulsion (Lipomul-IV, Upjohn, 15 per cent cottonseed oil) upon plasma clearance rate of fat.

The plasma clearance of fat was measured every 3 to 4 days during a two-week period in which 500 to 600 ml. of the fat emulsion was administered daily to 5 chronically ill patients. Seventy-five milliliters of the emulsion (11.25 Gm. of fat) was injected intravenously during a 10-minute period and serum turbidity and serum triglyceride levels were determined in plasma samples collected at the end of the injection and every 10 minutes for an hour. The clearance rates were calculated from these data.

In 4 of the 5 patients there was a two- to fourfold reduction of clearance rate after 8 to 10 daily infusions of 500 to 600 ml. of the fat emulsion. In 1 patient the clearance rate slowed after only 5 infusions. In 2 of the patients there was also an increase in serum turbidity and in serum triglyceride in the fasting state 18-20 hours after the Lipomul infusion had been completed.

These observations indicate that the mechanisms for removal of neutral fat from the plasma become less efficient as a large amount of fat is administered. This decrease in ability to clear hyperlipemia may be related to some of the toxic manifestations that have been reported after prolonged daily use of intravenous fat emulsions.

77. THE EFFECTS OF VARIOUS ENDOGENOUS PYROGENS ON CIRCULATING GRANULOCYTES

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(INTRODUCED BY CARL G. HARFORD, M.D.)

Endogenous pyrogens differ in many respects from bacterial pyrogens; they have been described in sterile leukocytic exudates and more recently in the serum of rabbits and dogs injected with bacterial pyrogens. While bacterial pyrogens are known to produce a marked leukopenia lasting at least an hour following intravenous injection, a similar phenomenon has not been demonstrated with endogenous pyrogens. The following experiments describe a much briefer and more prompt granulocytopenia following the injection of various endogenous pyrogens.

Serum containing endogenous pyrogen was collected from donor rabbits two hours after the injection of 1 ml. of undiluted typhoid vaccine. A 20 ml. aliquot of serum thus obtained was then injected intravenously into normal rabbits; the recipient's temperature and leukocyte count were followed closely. Equal amounts of normal serum were used for injection into animals serving as controls.

Within 5 minutes after the injection of serum containing endogenous pyrogen the total leukocyte count uniformly fell to approximately 40 per cent of the preinjection level. Differential counts revealed an almost complete absence of polymorphonuclear leukocytes during the period of maximal leukopenia. The leukocyte count rose to normal within 30 minutes and then to about twice the preinjection level after two hours. Normal serum, however, produced only a small drop in the leukocyte count followed by an insignificant rise. In a second series of experiments heparinized plasma was used instead of serum; the results were identical.

Extracts of polymorphonuclear leukocytes were tested and they also produced an immediate and marked leukopenia followed by a leukocytosis. However, supernatant fluid from sterile peritoneal exudates produced a similar leukocytosis without an initial leukopenia. Throughout this study fevers of similar magnitude were produced in each group of rabbits. For comparison bacterial pyrogen was given in doses small enough to produce equal fevers, but the leukopenia typically encountered with large amounts of endotoxin did not occur.

These findings suggest the possibility that the leukopenia that classically follows the injection of bacterial pyrogens is caused by the newly formed endogenous pyrogen rather than by the bacterial pyrogen itself.

78. ISOLATION OF DEHYDROEPIANDROSTERONE FROM MECONIUM

RALPH A. KINSELLA, JR., M.D., FAITH ELLEN FRANCIS, PH.D., AND NAI-HSUAN CHANG SHEN, PH.D., ST. LOUIS, MO.

(INTRODUCED BY RALPH A. KINSELLA, M.D.)

The steroid hormonal metabolism of the fetus seems to be different from both that of the pregnant woman and that of the adult. The principal 17-ketosteroid of meconium appears to be dehydroepiandrosterone (DHA) which has been isolated and identified. This metabolite, which is derived from an unknown precursor, may be absent from normal adult urine and from pregnancy urine, but is excreted in large amounts by patients with hyperadrenalcorticism. DHA was not found in pools of urine of the newborn infant (each of the first four days of life) but epiandrosterone, which is invariably found in adult urine, was found. Conversely, epiandrosterone has not been detected in meconium. Since DHA is excreted in urine and meconium as a sulfate, a phylogenetically earlier conjugate than glucuronide, it seems reasonable to relate this compound and its precursor more to the metabolic activity of the fetus than of the adult. While these observations indicate that steroid hormonal metabolism of the pregnant woman, the placenta, and the fetus may not be viewed as simply commingled, the extent to which the observed differences may be ascribed to differences in production rather than to differences in metabolic transformation is not clear. Perhaps either DHA, or its precursor, or the related intermediary metabolism is characteristic of the fetal period of life. Excretion of DHA by the adult, in whose urine this compound is a relatively small fraction of the total 17-ketosteroids, would be viewed as persistence of a fetal trait. The loss of differentiation characteristic of tumors would be consistent with excretion of increased amounts of DHA by patients with adrenal tumors. The precursor of DHA may be a major hormone of the fetal, in contrast to the adult, adrenal.

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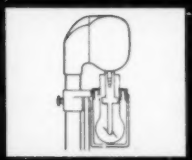

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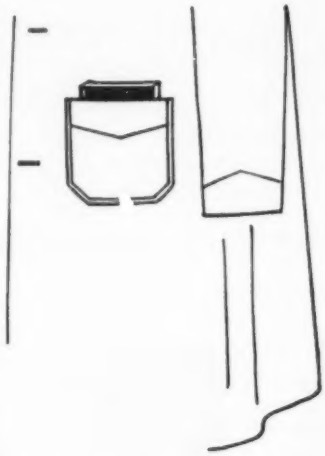

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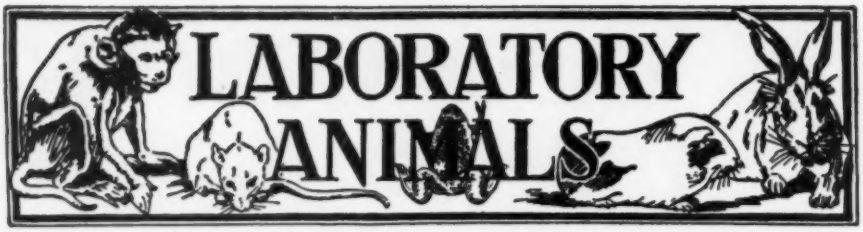

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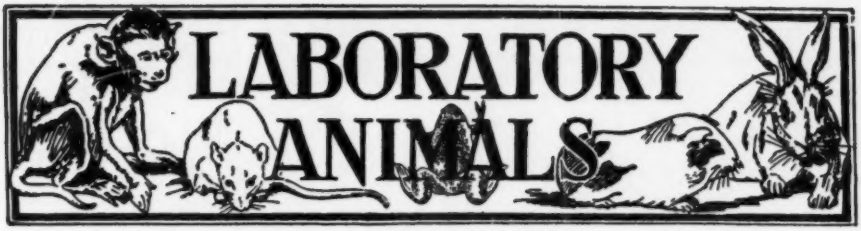

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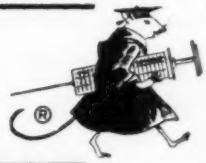

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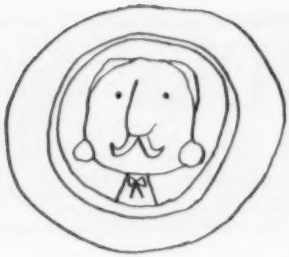

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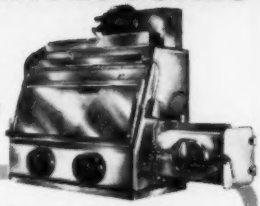

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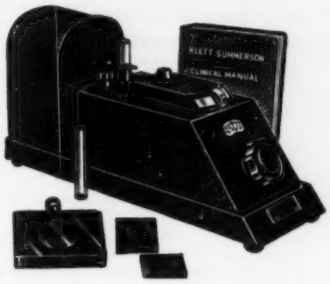

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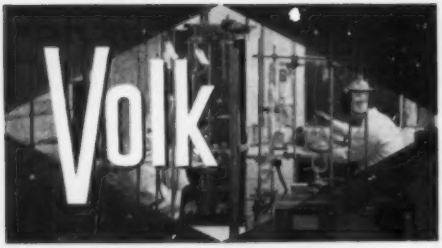

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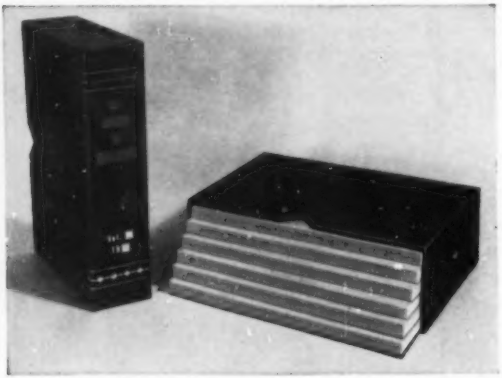

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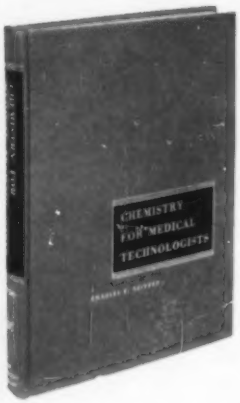

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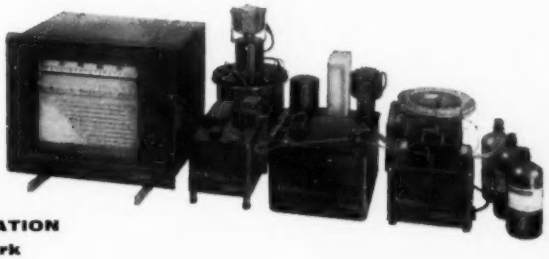

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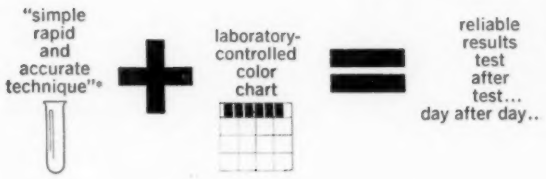
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